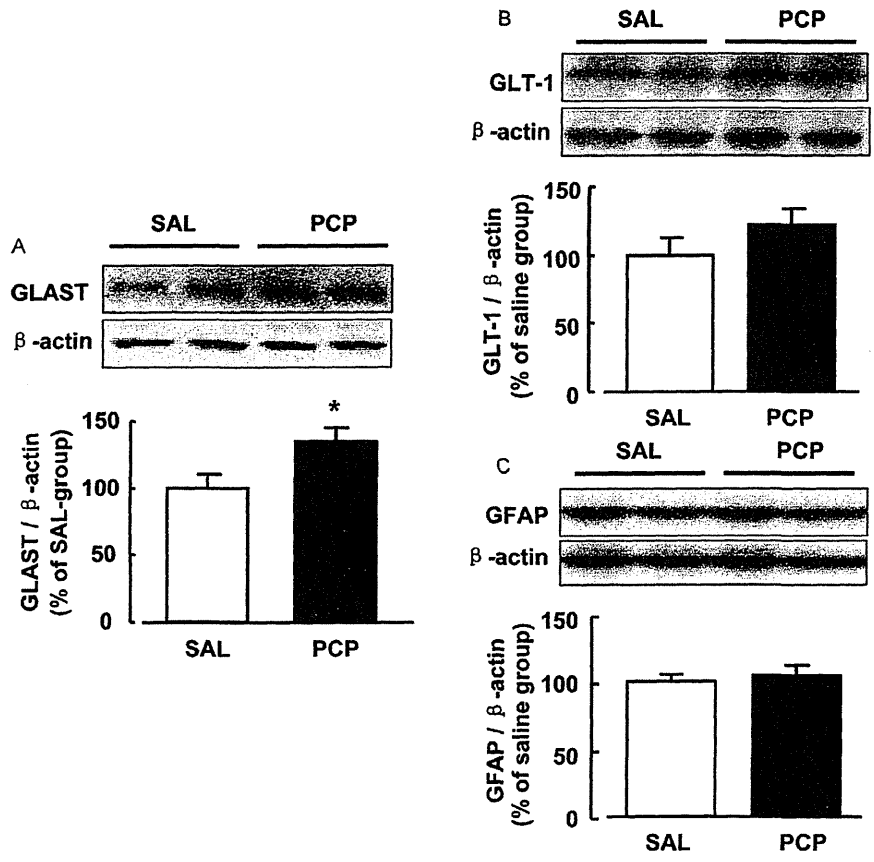


Fig. 4. Changes in the expression of glutamate transporters in the prefrontal cortex of prenatal PCP-treated mice. Representative Western blots band for the expression of GLAST, GLT-1 and GFAP. The amount of protein (30 μg/well) loaded was normalized to that of β-actin. Results are represented as the level of GLAST (A), and GLT-1 (B), as well as GFAP (C) in the prefrontal cortex. *$P<0.05$ compared with the prenatal SAL-treated group. Data are expressed as the mean ± S.E.M. for 6–7 mice in each group (Student's *t*-test). SAL, saline; PCP, phencyclidine.

higher dose of DL-TBOA (10 nmol) ($F_{group\,(1,40)}=24.66$, $P<0.01$; $F_{treatment\,(2,40)}=3.96$, $P<0.05$; $F_{group\times treatment\,(2,40)}=6.51$, two-way ANOVA; $P<0.01$, Fig. 5C). However, there were no significant differences in total exploration time in the retention session among each group ($F_{group\,(1,40)}=0.14$, $P>0.05$; $F_{treatment\,(2,40)}=0.02$, $P>0.05$; $F_{group\times treatment\,(2,40)}=1.27$ $P>0.05$; two-way ANOVA; Fig. 5D). These results suggested that DL-TBOA did not affect motivation or curiosity, but ameliorated the impairment of recognition memory in the prenatal PCP-treated mice.

Next, we evaluated the effects of DL-TBOA on the prolonged immobility time in the forced swimming test in the PCP-treated mice. DL-TBOA (10 nmol) significantly reversed the prolonged immobility induced by prenatal PCP exposure in the forced swimming test ($F_{group\,(1,40)}=18.03$, $P<0.01$; $F_{treatment\,(2,40)}=7.42$, $P<0.01$; $F_{group\times treatment\,(2,40)}=3.10$, $P=0.06$; two-way ANOVA; Fig. 5E), but did not change immobility time in the SAL-treated mice. These results indicated DL-TBOA to be effective in correcting emotional abnormalities induced by prenatal exposure to PCP without affecting motility.

Furthermore, we continued to evaluate the effects of DL-TBOA on the reduced extracellular glutamate level and impairment of K^+-induced glutamate release induced by prenatal PCP treatment. After the basal levels of glutamate reached a steady state, DL-TBOA was given through the probe for dialysis and the basal release of glutamate was monitored for 90 min. DL-TBOA tended to normalize the level of extracellular glutamate in the prenatal PCP-treated mice ($F_{(2,18)}=2.60$, $P=0.10$; one-way ANOVA; Fig. 6A). Moreover, it clearly improved the high K^+ (100 mM)-induced glutamate release reduced by prenatal exposure to PCP ($F_{group\,(2,18)}=9.09$, $P<0.01$; $F_{time\,(4,18)}=22.45$, $P<0.01$; $F_{group\times time\,(8,18)}=2.43$, $P<0.05$; repeated two-way ANOVA for 10–50 min; Fig. 6B). These results indicated that DL-TBOA reversed the reduction in glutamatergic neurotransmission observed in the prenatal PCP-treated mice.

4. Discussion

The blockade of NMDA receptors by PCP in the developing brain has been found to impair learning and memory. For instance, prenatal exposure to PCP disrupts passive avoidance and pole-climbing avoidance responses [36], and impairs performance in the eight-arm maze and Morris water maze in adult rats [2,51]. In the present study, prenatal PCP treatment produced an impairment of memory in the novel object recognition test, consistently suggested a cognitive deficit in this model. Furthermore, the prenatal PCP-treated mice showed a prolonged immobility in the forced swimming test, which are frequently observed in PCP animal models displaying schizophrenia-like negative symptom [38]. Taken together, these results indicate that the blockade of NMDA receptors by PCP in the prenatal period triggers cognitive and emotional abnormalities in postpubertal mice.

Glutamate neurotransmission plays a critical role in synaptic activity and plasticity throughout the brain, including cognition-, emotion- and reward-related circuits [31]. In schizophrenic patients, evidence of abnormal glutamatergic transmission has been found, such as disturbances of cortical glutamate release

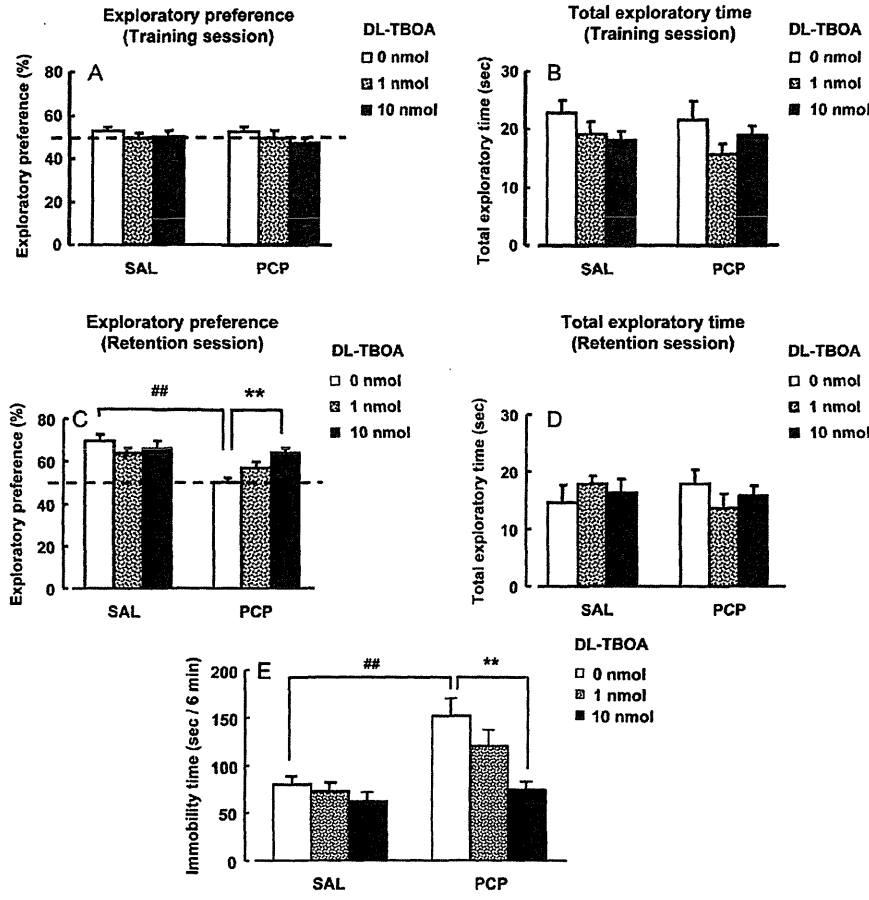


Fig. 5. Effects of DL-TBOA on the behavior in the prenatal PCP-treated mice. DL-TBOA (1 and 10 nmol) was administered by microinjection directly into the prefrontal cortex 30 min before each behavioral test. Exploratory preference (%) in the training session (A) and retention session (C). Total exploration time (s) in the training session (B) and retention session (D) of the novel object recognition test. Immobility time (s) was assessed for 6 min in the forced swimming test (E). Data are expressed as the mean ± S.E.M. for 7–8 mice in each group. $^{\#\#}P<0.01$ compared with the prenatal SAL-treated group; $^{*}P<0.05$, $^{**}P<0.01$ compared with the prenatal PCP-treated group (two-way ANOVA with Bonferroni's test). SAL, saline; PCP, phencyclidine.

[7,8,14,24], lower glutamate levels in the PFC [46], and decreased levels of glutamate in cerebrospinal fluid [19], as well as reduced glutamatergic tone in the cortex area [16]. In PCP-treated adult mice, a decrease in spontaneous extracellular glutamate release [34] and in the level of phosphorylated-NR1 [33,34], but an increase in levels of GLAST expression has been observed in the PFC [34]. In the present study, we found a decrease in both the extracellular glutamate concentration and high K^+-induced release of glutamate in the PFC of the PCP-treated mice compared with the SAL-treated mice, suggesting that prenatal exposure to PCP produced a pre-

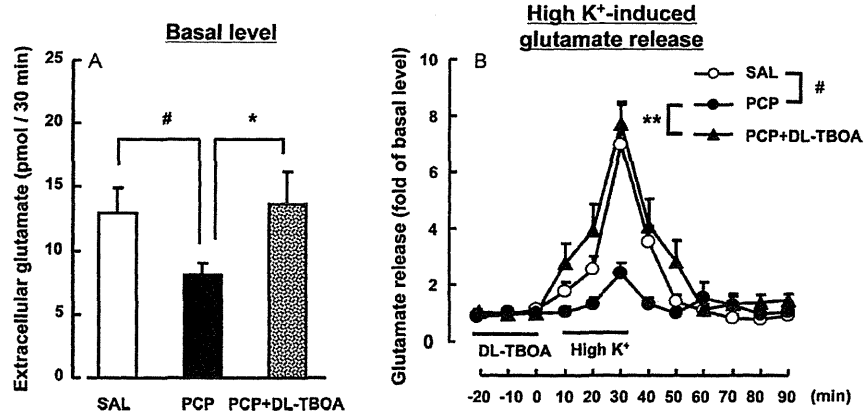


Fig. 6. Effects of DL-TBOA on the reduction of glutamate release in the prenatal PCP-treated mice. DL-TBOA (1 mM) was administered through a micodialysis tube into the prefrontal cortex of mice for 30 min (μl/min). After the administration, basal glutamate release (A) and K^+-evoked (100 mM) glutamate release (B) in the prefrontal cortex of prenatal SAL- or PCP-treated mice were determined. Data are expressed as the mean ± S.E.M. for 7 mice in each group. $^{\#}P<0.05$ compared with the prenatal SAL-treated group; $^{*}P<0.05$, $^{**}P<0.01$ compared with the prenatal PCP-treated group (one-way ANOVA or repeated two-way ANOVA with Bonferroni's test). SAL, saline; PCP, phencyclidine.

synaptic hypofunctional glutamatergic neurotransmission. Given that glutamate neurotransmission plays a critical role in synaptic plasticity and long-term potentiation [31], it is possible that the hypofunctional glutamatergic neurotransmission was associated with these behavioral deficits induced by prenatal PCP treatment.

Glutamate transporters, GLAST and GLT-1, are considered to regulate glutamate transmission by rapidly clearing glutamate from extracellular fluid [11]. It is suggested that an increase in GLAST expression contributes to a decrease in extracellular glutamate release, because the regulation of extracellular glutamate levels by membrane transporters is important for terminating synaptic transmission in the brain [43]. Furthermore, increased numbers of binding sites and protein expression of glutamate transporters have been observed in the postmortem brain of schizophrenia patients [9,11,19,37,42]. Additionally, antipsychotics such as clozapine inhibites the increase in glutamate transporters [29,48]. In this study, we observed the overexpression of GLAST protein in the prenatal PCP-treated mice, although no significant change in GLT-1 expression. GLAST is produced during embryogenesis in rodents, while GLT-1 is expressed in the forebrain postnatally [47]. Therefore, prenatal exposure to PCP might lead to different patterns of GLAST and GLT-1 expression. Furthermore, we found that DL-TBOA, a potent glutamate transporter blocker, attenuated the cognitive and emotional deficits by normalizing the extracellular release of glutamate. These results suggested that the elevated expression of GLAST protein was, at least in part, responsible for the dysfunctional glutamate transmission associated with these behavioral changes in the prenatal PCP-treated mice.

GLAST proteins are expressed in glial cells of the adult brain and spinal cord [23,41]. However, in this study, we failed to detect a significant change in the expression of GFAP, a marker of glial cells. Thus, it is unlikely that the increase in GLAST expression is due to the activation of glial cells. It was reported that Ca^{2+} influx inhibits GLAST expression in astrocytes [26]. Since PCP inhibited the influx of Ca^{2+} by blocking the NMDA receptor, one possibility is that the disruption of Ca^{2+} influx into astrocytes is associated with the up-regulation of GLAST expression. Furthermore, any factors which enhance the gene transcription of GLAST or disrupt the protein's degradation might also contribute to the up-regulated expression. The precise mechanism remains to be elucidated.

The NMDA receptors are thought to control the differentiation and migration of immature neurons [4,20]. Many neurons undergo a stage when they are critically dependent on stimulation by glutamate through the NMDA receptors, and sustained deprivation of this input by NMDA receptor antagonists during development activates apoptosis [17]. Therefore, we could not exclude the possibility that a loss of glutamatergic neurons contributes to the glutamatergic hypofunction, although here, we did not observe any significant decrease in the total number of neurons in adult mice. Thus, any neurodevelopmental disturbances caused by prenatal exposure to PCP in development are potentially implicated in these behavioral and biochemical changes.

5. Conclusion

The present findings indicate that prenatal exposure to PCP leads to cognitive impairment and emotional dysfunction, which are accompanied by a disruption to pre-synaptic glutamate neurotransmission through the enhanced expression of glutamate transporters in the PFC. Since the abnormal glutamatergic release and the altered expression of glutamate transporters are involved in the pathophysiology of schizophrenia, this study provides further insights into how psychiatric illnesses develop.

Conflicts of interest

The authors state no conflict of interest.

Acknowledgements

This study was supported by Grants-in-aid for Scientific Research (A) (22248033), Scientific Research (B) (20390073) (21390045) and Exploratory Research from the JSPS (19659017) (22659213) by the 'Academic Frontier' Project for Private Universities (2007–2011) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT); by Regional Joint Research Program supported by grants to Private Universities to Cover Current Expenses from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), by Research on Regulatory Science of Pharmaceuticals and Medical Devices from the Ministry of Health and Labour and Welfare (MHLW); by Research on Risk of Chemical Substances, Health and Labour Science Research Grants supported by the Ministry of Health, Labour and Welfare (MHLW); by the joint research project under the Japan-Korea basic scientific cooperation program by Japan Society for the Promotion of Science (JSPS); by the Brain Research Center from 21st Century Frontier Research Program supported by the Ministry of Science and Technology, Republic of Korea.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbr.2011.01.035.

References

[1] Abekawa T, Ito K, Nakagawa S, Koyama T. Prenatal exposure to an NMDA receptor antagonist, MK-801 reduces density of parvalbumin-immunoreactive GABAergic neurons in the medial prefrontal cortex and enhances phencyclidine-induced hyperlocomotion but not behavioral sensitization to methamphetamine in postpubertal rats. Psychopharmacology (Berl) 2007;192:303–16.
[2] Andersen JD, Pouzet B. Spatial memory deficits induced by perinatal treatment of rats with PCP and reversal effect of D-serine. Neuropsychopharmacology 2004;29:1080–90.
[3] Ashdown H, Dumont Y, Ng M, Poole S, Boksa P, Luheshi GN. The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. Mol Psychiatry 2006;11:47–55.
[4] Behar TN, Scott CA, Greene CL, Wen X, Smith SV, Maric D, et al. Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. J Neurosci 1999;19:4449–61.
[5] Belforte JE, Zsiros V, Sklar ER, Jiang Z, Yu G, Li Y, et al. Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. Nat Neurosci 2010;13:76–83.
[6] Bellinger FP, Wilce PA, Bedi KS, Wilson P. Long-lasting synaptic modification in the rat hippocampus resulting from NMDA receptor blockade during development. Synapse 2002;43:95–101.
[7] Carlsson A, Hansson LO, Waters N, Carlsson ML. Neurotransmitter aberrations in schizophrenia: new perspectives and therapeutic implications. Life Sci 1997;61:75–94.
[8] Carlsson A, Waters N, Carlsson ML. Neurotransmitter interactions in schizophrenia-therapeutic implications. Biol Psychiatry 1999;46:1388–95.
[9] Chen L, Yang CR. Interaction of dopamine D1 and NMDA receptors mediates acute clozapine potentiation of glutamate EPSPs in rat prefrontal cortex. J Neurophysiol 2002;87:2324–36.
[10] D'Souza SW, McConnell SE, Slater P, Barson AJ. Glycine site of the excitatory amino acid N-methyl-D-aspartate receptor in neonate and adult brain. Arch Dis Child 1993;69:212–5.
[11] Danbolt NC. Glutamate uptake. Prog Neurobiol 2001;65:1–105.
[12] Deutsch SI, Mastropaolo J, Rosse RB. Neurodevelopmental consequences of early exposure to phencyclidine and related drugs. Clin Neuropharmacol 1998;21:320–32.
[13] du Bois TM, Huang XF. Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia. Brain Res Rev 2007;53:260–70.
[14] Frankle WG, Lerma J, Laruelle M. The synaptic hypothesis of schizophrenia. Neuron 2002;39:205–16.
[15] Franklin JB, Paxinos GT. The mouse brain. In: Stereotaxic coordinates. New York: Academic; 1997.
[16] Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 2001;158:1367–77.

[17] Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283:70–4.
[18] Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991;148:1301–8.
[19] Kim JS, Kornhuber HH, Schmid-Burgk W, Holzmüller B. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. Neurosci Lett 1980;20:379–82.
[20] Komuro H, Rakic P. Modulation of neuronal migration by NMDA receptors. Science 1993;260:95–7.
[21] Lahti AC, Koffel B, LaPorte D, Tamminga CA. Subanesthetic doses of ketamine stimulates psychosis in schizophrenia. Neuropsychopharmacology 1995;13:9–19.
[22] Lee E, Williams Z, Goodman CB, Oriaku ET, Harris C, Thomas M, et al. Effects of NMDA receptor inhibition by phencyclidine on the neuronal differentiation of PC12 cells. Neurotoxicology 2006;27:558–66.
[23] Lehre KP, Levy LM, Otterson OP, Storm-Mathisen J, Danbolt NC. Differential expression of two glutamate transporters in the rat brain: quantitative and immunohistochemical observations. J Neurosci 1995;15:1835–53.
[24] Lewis DA, Glantz LA, Pierri JN, Sweet RA. Altered cortical glutamate neurotransmission in schizophrenia: evidence from morphological studies of pyramidal neurons. Ann N Y Acad Sci 2003;1003:102–12.
[25] Liu J, Suzuki T, Seki T, Namba T, Tanimura A, Arai H. Effects of repeated phencyclidine administration on adult hippocampal neurogenesis in the rat. Synapse 2006;60:56–68.
[26] Liu YP, Yang CS, Tzeng SF. Inhibitory regulation of glutamate aspartate transporter (GLAST) expression in astrocytes by cadmium-induced calcium influx. J Neurochem 2008;105:137–50.
[27] Lu L, Mamiya T, Lu P, Toriumi K, Mouri A, Hiramatsu M, et al. Prenatal exposure to phencyclidine produces abnormal behaviour and NMDA receptor expression in postpubertal mice. Int J Neuropsychopharmacol 2010;13:877–89.
[28] Maddox VH, Godefroi EF, Parcell RF. The synthesis of phencyclidine and other 1-arylcyclohexylamines. J Med Chem 1965;8:230–5.
[29] Melone M, Vitellaro-Zuccarello L, Vallejo-Illarramendi A, Perez-Samartin A, Matute C, Cozzi A, et al. The expression of glutamate transporter GLT-1 in the rat cerebral cortex is down-regulated by the antipsychotic drug clozapine. Mol Psychiatry 2001;6:380–6.
[30] Millan MJ. N-methyl-D-aspartate receptors as a target for improved antipsychotic agents: novel insight and clinical perspectives. Psychopharmacology (Berl) 2005;179:30–53.
[31] Moghaddam B. Bringing order to the glutamate chaos in schizophrenia. Neuron 2003;40:881–4.
[32] Mouri A, Noda Y, Enomoto T, Nabeshima T. Phencyclidine animal models of schizophrenia: approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. Neurochem Int 2007;51:173–84.
[33] Mouri A, Noda Y, Noda A, Nakamura T, Tokura T, Yura Y, et al. Involvement of a dysfunctional dopamine-D1/N-methyl-D-aspartate-NR1 and Ca^{2+}/calmodulin-dependent protein kinase II pathway in the impairment of latent learning in a model of schizophrenia induced by phencyclidine. Mol Pharmacol 2007;71:1598–609.
[34] Murai R, Noda Y, Matsui K, Kamei H, Mouri A, Matsuba K, et al. Hypofunctional glutamatergic neurotransmission in the prefrontal cortex is involved in the emotional deficit induced by repeated treatment with phencyclidine in mice: implications for abnormalities of glutamate release and NMDA-CaMKII signaling. Behav Brain Res 2007;180:152–60.
[35] Murray RM, O'Callaghan E, Castle DJ, Lewis SW. A neurodevelopmental approach to the classification of schizophrenia. Schizophr Bull 1992;18:319–32.
[36] Nabeshima T, Hiramatsu M, Yamaguchi K, Kasugai M, Ishizaki K, Kawashima K, et al. Effects of prenatal administration of phencyclidine on the learning and memory processes of rat offspring. J Pharmacobiodyn 1988;11:816–23.
[37] Nanitsos EK, Nguyen KT, St'astny F, Balcar VJ. Glutamatergic hypothesis of schizophrenia: involvement of Na^+/K^+-dependent glutamate transport. J Biomed Sci 2005;12:975–84.
[38] Noda Y, Yamada K, Furukawa H, Nabeshima T. Enhancement of immobility in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia. Br J Pharmacol 1995;116:2531–7.
[39] Pilowski LS, Kerwin RW, Murray RM. Schizophrenia: a neuro-developmental perspective. Neuropsychopharmacology 1993;9:83–91.
[40] Scheetz AJ, Constantine-Paton M. Modulation of NMDA receptor function: implications for vertebrate neural development. FASEB J 1994;8:745–52.
[41] Schmitt A, Asan E, Puschel B, Kugler P. Cellular and regional distribution of the glutamate transporter GLAST in the CNS of rats: nonradioactive in situ hybridization and comparative immunocytochemistry. J Neurosci 1997;17:1–10.
[42] Simpson MD, Slater P, Deakin JF. Comparison of glutamate and gamma-aminobutyric acid uptake binding sites in frontal and temporal lobes in schizophrenia. Biol Psychiatry 1998;44:423–37.
[43] Takahashi M, Billups B, Rossi D, Sarantis M, Hamann M, Attwell D. The role of glutamate transporters in glutamate homeostasis in the brain. J Exp Biol 1997;200:401–19.
[44] Tanimura A, Liu J, Namba T, Seki T, Matsubara Y, Itoh M, et al. Prenatal phencyclidine exposure alters hippocampal cell proliferation in offspring rats. Synapse 2009;63:729–36.
[45] Theiler K. The house mouse-atlas of embryonic development. New York: Springer-Verlag; 1989.
[46] Tsai G, Passani LA, Slusher BS, Carter R, Baer L, Kleinman JE, et al. Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. Arch Gen Psychiatry 1995;52:829–36.
[47] Ullensvang K, Lehre KP, Storm-Mathisen J, Danbolt NC. Differential developmental expression of the two rat brain glutamate transporter proteins GLAST and GLT. Eur J Neurosci 1997;9:1646–55.
[48] Vallejo-Illarramendi A, Torres-Ramos M, Melone M, Conti F, Matute C. Clozapine reduces GLT-1 expression and glutamate uptake in astrocyte cultures. Glia 2005;50:276–9.
[49] Wang C, Anastasio N, Popov V, Leday A, Johnson KM. Blockade of N-methyl-aspartate receptors by phencyclidine causes the loss of corticostriatal neurons. Neuroscience 2004;125:473–83.
[50] Wang C, McInnis J, Ross-Sanchez M, Shinnick-Gallagher P, Wiley JL, Johnson KM. Long-term behavioral and neurodegenerative effects of perinatal phencyclidine administration: implications for schizophrenia. Neuroscience 2001;107:535–50.
[51] Yanai J, Avraham Y, Levy S, Maslaton J, Pick CG, Rogel-Fuchs Y, et al. Alterations in septohippocampal cholinergic innervations and related behaviors after early exposure to heroin and phencyclidine. Brain Res Dev Brain Res 1992;69:207–14.

1358 *Biol. Pharm. Bull.* 34(9) 1358—1363 (2011) Vol. 34, No. 9

Current Topics

Translational Research in Neurodevelopmental Disorders: Development of Etiology-Based Animal Models

Genetic Animal Models of Schizophrenia Related with the Hypothesis of Abnormal Neurodevelopment

Lingling Lu,[a,b] Takayoshi Mamiya,[a,b,c] Takenao Koseki,[a,b] Akihiro Mouri,[a,b,c] and Toshitaka Nabeshima*,[a,b,c]

[a] *The Academic Frontier Project for Private University, Comparative Cognitive Science Institute, Meijo University;* [b] *Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University; Nagoya 468–8503, Japan: and* [c] *Japanese Drug Organization for Appropriate Use and Research; Nagoya 468–0069, Japan.*

Received January 4, 2011

Accumulating evidence supports the existence of an overlap in genetic susceptibility with schizophrenia. Translation of human genetic mutations into animals is one of the most important strategies to study the pathogenesis of schizophrenia, identify potential drug targets, and test new medicines for antipsychotic treatment. Recent discoveries of susceptibility genes for schizophrenia make the possibility to develop newer genetic mouse models based on the neurodevelopmental hypotheses of schizophrenia. Although it is not possible to mimic all schizophrenic symptoms by these animal models, the genetic mouse models based on the neurodevelopmental hypothesis are widely developed to reproduce several schizophrenia-like behavioral and biochemical changes in humans. In this mini review, we will discuss the neuropathological and behavioral manifestations of representative genetic mouse models for schizophrenia, associated with the hypothesis of abnormal neurodevelopment.

Key words schizophrenia; genetic mouse model; neurodevelopmental hypothesis

1. INTRODUCTION

Schizophrenia is a heritable mental disorder characterized by chronic psychotic symptoms and cognitive deficits. For more than three decades, the neurodevelopmental hypothesis has prevailed for schizophrenia. This hypothesis posits that schizophrenia is the behavioral outcome of an aberration in neurodevelopmental processes that begins long before the onset of clinical symptoms[1,2] and involves deficits in the genetic program for normal formation of synapses and migration of neurons, as well as their connections in brain development.[3]

With growing evidences on the neurobiology and genetics of schizophrenia, more animal models have been developed to study the molecular mechanisms of pathophysiological changes and to design more effective therapies for schizophrenia. In principle, genetic manipulation offers advantages over pharmacologic models because it is more selective in its molecular targets, it is developmental by nature, and it can be varied in terms of penetrance, allele dose, and temporal characteristics.[4] Genes involved in regulation of cortical cytoarchitecture during development have been suspected to mediate abnormal neurodevelopment in schizophrenia.[5—7] Therefore some of these genes have been employed to make potential animal models of schizophrenia.

In this article, we briefly review the genetic animal models related with the hypothesis of abnormal neurodevelopment of schizophrenia at present.

2. GENETIC EVIDENCE OF NEURODEVELOPMENTAL HYPOTHESIS

Schizophrenia is a clinically heterogeneous psychotic illness whose etiology remains poorly understood. However, clinical, epidemiological, genetic, and neuropathological features of schizophrenia continue to suggest that abnormal neurodevelopment is important for the disorder.[8] Genetic studies have identified several specific genes that are associated with schizophrenia risk in a number of populations. Generally, twin studies have shown that schizophrenia is a predominant genetic disorder, with estimates of heritability risk ranging at 50—60% and there are recent reports of 80%.[9—12] Family studies have found that single effects of a major gene are unlikely; instead, polygenic models effects of multiple-risk genes acting additively or multiplicatively may provide the best explanation for schizophrenia.[13] Several neurodevelopment-related genes are located in chromosomal loci that are associated with potential candidate genes due to their polymorphic status, and to alter their expression during embryonic stages. They might putatively result in the neurodevelopmental abnormalities observed in schizophrenia.[14] Hence identification of genes responsible for this high heritability will be critical to understanding this disorder. Moreover, without parsing based on genotypes, the environmental and epigenetic factors may be more difficult to clarify.

Anatomical abnormalities such as ventricular enlargement, volume reductions of prefrontal cortex and hippocampus, and generalized brain reduction are well established among other features of schizophrenia.[15—17] Obvious alterations in neuron size and morphology as well as synaptic connectivity are also observed in schizophrenic patients.[18] Investigations focusing on cortical and limbic brain regions increasingly demonstrate that structural and molecular integrity of the synaptic complex, glutamate-related receptors, and signal transduction pathways take critical roles in brain develop-

* To whom correspondence should be addressed. e-mail: tnabeshi@meijo-u.ac.jp

ment, synaptogenesis, and synaptic plasticity.[8] More importantly, most schizophrenia-related specific genes including disrupted in schizophrenia-1 (DISC1), neuregulin-1 (NRG1), dysbindin-1, and AKT-1 play important roles in neurodevelopment, neurotransmission, and neuroplasticity. Although variations among each gene confer only modest increases in the risk for schizophrenia, the discovery of these genes and identification of candidate proteins and molecular pathways may importantly contribute to the pathophysiology of schizophrenia.[8]

3. GENETIC ANIMAL MODELS RELATED TO THE NEURODEVELOPMENTAL HYPOTHESES OF SCHIZOPHRENIA

Schizophrenia-Susceptibility Genes. Disrupted in Schizophrenia-1 (DISC1) Schizophrenia susceptibility genes have been identified by human genetic studies and enabled us to generate mouse models on the basis of genetic etiology. Because causal mutations have not been identified, there is still debate on the significant roles of each gene. Nonetheless, many of the genetically engineered models for these genes display behavioral abnormalities and morphological alterations that may be relevant to schizophrenia patients. Here, we summarize the behavioral and morphological changes in representative genetic mouse models that are associated with the neurodevelopmental hypotheses of schizophrenia.

Numerous studies have found that DISC1 is highly expressed during brain development and plays critical roles in the growth of the embryonic and postnatal brain.[19,20] It has been confirmed that DISC1 affects the development and maturation of neuronal systems, which is implicated with psychiatric disorders.[21] DISC1 was originally identified as truncated by a translocation that segregated with schizophrenia. Therefore several transgenic animal models expressing this truncated protein have been generated. In several kinds of transgenic mice expressing truncated DISC1, enlarged lateral ventricles,[22,23] reduced cortical thickness, and partial agenesis in the corpus callosum[24] as well as reduced immunoreactivity of parvalbumin (PV) in both the medial prefrontal cortex (mPFC) and the hippocampus[22,24] have been detected as important hallmarks for schizophrenia. Furthermore, transient expression of a dominant-negative DISC1 (C-terminal fragment of DISC1) at postnatal day 7 (PD 7), produces a reduction of hippocampal synaptic transmission[25] as a result of decrease of the hippocampal dendritic complexity. In another type of DISC1 transgenic mice, misorientated and shorter dendrites and decrease in numbers of synaptic spines of hippocampal granule cells[26] have been found, which cause reduced short-term potentiating at CA3/CA1 synapses and indirect working memory deficit.[26]

Recent study has revealed critical roles of DISC1 in regulation of the embryonic and adult neurogenesis.[27] DISC1 is highly expressed in the embryonic ventricular/subventricular zones of the cortex where neural progenitor cells reside, suggesting that this gene regulates their proliferation and/or differentiation. To confirm this idea, DISC1 RNA interference (RNAi) was introduced into neural progenitors in the developing neocortex, using *in utero* electroporation, and a significant reduction of proliferation of progenitor cells and differentiation of premature neurons was observed. Interestingly, overexpression of DISC1 in neural progenitors results in an opposite phenotype. It has been also found that knockdown of DISC1 using a lentivirus to deliver RNAi results in a decrease in the proliferation of adult progenitor cells in the dentate gyrus.[27] Moreover, such transgenic DISC1 mutant mice show hyperlocomotion in a novel environment and increased immobility time in a forced swimming test, which are frequently observed in animal models displaying schizophrenia-like behavior.[27]

In our recent study, by utilizing the methods of in utero electroporation, we have successfully generated a novel mouse model by transferring RNAi of DISC1 at embryonic day 14 (E 14) selectively to disrupt DISC1 gene expression in a lineage for pyramidal neurons mainly in the prefrontal cortex during neurodevelopment.[28] Our results indicate that knockdown of DISC1 leads to maturation-dependent deficits in mesocortical dopaminergic projections and induces a series of schizophrenia-like behavioral abnormalities including hyperlocomotion in a novel environment, enhanced immobility time in forced swimming test, deficits in prepulse inhibition (PPI) of startle response, and memory impairments in novel object recognition test after sexual maturation. Importantly, these behavioral abnormalities are attenuated by treatment with atypical antipsychotic clozapine, indicating their association with schizophrenia.[28] Moreover, we further observed a significant decrease in the extracellular level of dopamine and tyrosine hydroxylase (TH), a marker of mature axonal terminals of the dopaminergic projection, and disturbances of PV interneurons and pyramidal neurons in mPFC of DISC1 knockdown mice at PD 56 but not PD 28 or PD 42.[28] These results suggest that DISC1 may play critical roles in neurodevelopment, and its disruption during development may induce several schizophrenia-like features in adult mice.

Although disruption of DISC1 may be critically involved in many cases of schizophrenia, direct evidence from genetic linkage and association studies suggests that variants of DISC1 may not occur in most cases of the disorder, compared with other psychotic diseases.[21]

Neuregulin-1 (NRG-1) NRG-1 was first identified as a susceptibility gene for schizophrenia in an Icelandic population[29] and further confirmed by subsequent studies.[30,31] NRG-1 plays important roles in brain development, such as neuronal migration and neurite outgrowth, as well as proliferation of glia cells.[32] Furthermore, increased levels of NRG-1 type I mRNA are observed in schizophrenia patients.[33,34] Tissue culture study has revealed that NRG-1 dampens *N*-methyl-D-aspartate (NMDA) receptor function in pyramidal neurons of the prefrontal cortex.[35] Therefore NRG-1 mutant is considered a useful in genetic animal model for schizophrenia.

NRG-1 transgenic mouse models have been generated by manipulation of expression of different NRG1 isoforms by several studies. Several schizophrenia-like behaviors, such as hyperlocomotor activity and impaired PPI, as well as decreased expression of NMDA receptor have been observed in heterozygous NRG-1 knockout mice lacking the transmembrane domain of NRG-1 gene.[29] Moreover, disruption of type III NRG1 in adult mice results in increased volume of lateral ventricles and decreased density of dendritic spines in

hippocampal pyramidal neurons.[36] Knockout of the NRG-1 receptors ErbB2 and ErbB4 (ErbB2/4) at early embryonic stage decreases the density of spine in both the cortex and hippocampus.[37] In behavioral analysis, ErbB2/4 knockout mice display an increase in aggression and a deficit in PPI, as a model of sensorimotor gating that is abnormal in schizophrenia patients.[37] Moreover, ErbB4 knockout mice show a decrease in the power of kainate-induced gamma oscillations[38] and reduction of the density of calbindin-positive GABAergic interneurons in the cortex, as well as PV-positive interneurons in the hippocampus.[39]

Most positive single nucleotide polymorphisms (SNPs) are located upstream of the start site of NRG-1 exons, which suggests a probable effect on its expression. Whereas, the consequences of mutations in the region of NRG-1 remain unknown, and whether the heterozygotes knockout resembles a disease state is arguable. In addition, NRG-1 comprises in at least six major isoforms and many splice variants. The alternations of which one is particularly important for schizophrenia remain unclear, but they are quite critical to design genetically engineered animal models related to this disorder.

Dysbindin Dysbindin is another likely susceptibility gene that has been identified by several studies.[40,41] Irish study of high-density schizophrenia families has suggested that schizophrenic patients with negative symptoms are more likely to inherit the risk of dysbindin mutant, raising the possibility that negative symptoms in psychotic bipolar cases of schizophrenia are likely attributable to heritability of dysbindin mutation. Postmortem study has reported a decrease in the level of gene transcription of dysbindin and its protein expression in brain tissues of schizophrenia patients.[42]

Thus sandy (Sdy) mouse has been designed to mimic a deficiency of dysbindin in human.[43] As reported, Sdy mouse harbors a spontaneously occurring deletion in the DTNBP1 gene and expresses no dysbindin protein, which provides a unique tool to study the role of dysbindin in schizophrenia. Sdy mice also exhibit morphological changes in excitatory asymmetrical synapses on hippocampal CA1 dendritic spines, larger vesicle size, slower vesicle release, and lower release probability, as well as smaller total population of the readily releasable vesicle pool.[44] These mutant mice display deficits of neurosecretion and synaptic morphology in hippocampal neurons, and manifest some schizophrenia-like behavior such as social withdrawal and cognitive deficits. In the hippocampus of Sdy mice, the level of Snapin (a SNAP25-binding protein and a synaptic priming regulator) is reduced, which suggests that destabilization of Snapin in the Sdy mice may lead to abnormal neurotransmission and abnormal behavior.[45] Although more information for the associations between dysbindin gene and schizophrenia is needed, Sdy mice are able to serve as a genetic animal model to identify potential pathways of dysbindin in schizophrenia.

Brain-Derived Neurotrophic Factor (BDNF) BDNF has been found to play important roles in promoting and modifying growth, development, survival of neuronal populations, and activity-dependent neuronal plasticity.[46] BDNF is implicated in the pathogenesis of schizophrenia, since its expression is reduced in some postmortem brains of schizophrenia patients,[47,48] indicating that the downregulated expression of BDNF leads to abnormalities in developing brain. Atypical BDNF knockout mice, in which one allele of BDNF gene is disrupted through the whole developmental stages, exhibit hyperactivity in locomotion and behavioral deficits in spatial learning and memory.[49,50] Dysfunction of non-spatial associative memory is also observed in conditional BDNF knockout mice, in which BDNF gene is disturbed approximately 3 weeks.[51] However, these mutant mice do not show hypersensitivity or deficits in PPI and fear conditioning.[52,53] Moreover, dysfunction of context-dependent fear memory has been found in inducible BDNF knockout mice, in which the disrupted BDNF gene is limited in specific brain regions at certain developmental stages.[54] Further inducible knockout of BDNF from the embryonic stage induces severer context-dependent memory deficits compared with later knockout mice. These data suggest that BDNF plays a critical role in neurodevelopment. However, the genetic linkage between BDNF and schizophrenia is relatively weak, although the biochemical and behavioral changes in BDNF knockout mice resemble several pathological changes of schizophrenia.[55,56]

Reelin Reelin is a glycoprotein that guides neurons and radial glial cells to corrected position in the developing brain. A series of studies suggest that reelin might be a vulnerability gene involved in the development of psychosis including schizophrenia.[57—59] "Reeler mice" is a naturally occurring mutant mice model generated by disrupting the reelin gene. These mice exhibit decreased expression of reelin and glutamic acid decarboxylase 67 (GAD67), as well as lower density of dendritic spine,[57] which resemble some pathological changes of schizophrenic patients.[58] Moreover, these mutant mice show behavioral deficits in PPI, which is specifically involved in schizophrenia, although the reelin mutant mice do not show abnormalities in working memory or social interaction. Nonetheless, the reelin gene appears to play important roles in neuronal development and could mediate outcomes of some causative mutations in other genes and critical environmental insults.

NMDA Receptor Subunit 1 (NR1) Genetic disruption of NR1 by traditional and conditional knockout in mice results in hyperlocomotion, stereotypy, abnormal social behavior, cognitive dysfunction, and abnormal brain development.[60—62] These abnormalities resemble several aspects of schizophrenia. Importantly, some deficits are attenuated by antipsychotic treatment. Roles of NR1 in the pathology of schizophrenia are further supported by decreased expression of NR1 in postmortem tissues from schizophrenic patients and an increase in NR1 expression by chronic antipsychotic treatment.[63—65] Small molecules that enhance the function of NMDA receptor are being tested as novel adjunct therapies for schizophrenia treatment in clinical trials.[66,67]

Recently, Belforte *et al.*[68] characterized a mouse strain in which the essential NR1 subunit of the NMDA receptor is selectively eliminated by 40—50% in cortical and hippocampal interneurons in early postnatal development. Consistent with the NMDA receptor hypofunction hypothesis of schizophrenia, the postnatal NR1-ablated mice exhibit distinct schizophrenia-related symptoms after adolescence, including novelty-induced hyperlocomotion, mating and nest-building deficits, as well as anhedonia-like and anxiety-like behaviors. In addition, impairment of social memory, spatial working memory, and prepulse inhibition are also observed in the mutant mice. Furthermore, reduced expression of GAD67 and

PV is accompanied by disinhibition of cortical excitatory neurons and reduced neuronal synchrony. However, postadolescent deletion of NR1 did not result in such abnormalities, suggesting that early postnatal inhibition of NMDA receptor activity in corticolimbic GABAergic interneurons contributes to the pathophysiology of schizophrenia-related disorders.[68]

Others Studies have focused on a number of interesting candidate genes in neurodevelopmental hypotheses of schizophrenia, such as neural cell adhesion molecule (NCAM), cyclin-dependent kinase-5 (CDK5), V-akt murine thymoma viral oncogene homolog 1 (Akt1), Lis1, and Lhx5 so as to prepare potential genetic models.[69—72] Mice expressing these mutant genes show abnormal development in the brain and some schizophrenia-like behavior, although the patterns of abnormality vary. Clearly, the potential genetically to manipulate genes that affect brain development and to explore phenomenological links to the molecular and behavioral phenotypes related to schizophrenia is virtually limitless.

4. PERSPECTIVES

Schizophrenia has been long recognized as a heritable mental illness that probably involves multiple genes with relatively modest effects across large populations.[73] Most of these identified susceptibility genes such as DISC-1, NRG-1, AKT1, and Reelin are known to have essential functions in neurodevelopment including neuronal differentiation, migration, survival, synaptogenesis, and apoptosis.[27,74—77] Thus some aspects of altered brain development in schizophrenia may be attributable to abnormal expression of genes that are essential for early neurodevelopmental processes. Furthermore, such genetic mechanisms may significantly interact with prenatal and/or perinatal environmental insults to enhance the risk of developing schizophrenia.

Until now, there is still debate whether it is possible to use rodent models to reflect psychiatric disorders in humans. However, genetically engineered mice in which susceptibility genes are modified have potential advantages over human studies. In the case of schizophrenia, initial risks for this disorder occur during neurodevelopment, whereas onset of the disease arises in adulthood, with almost two decades for the full development of pathology to overt incidence. To understand in detail the mechanisms of schizophrenia, it is important to characterize how the disorder etiologies develop over time until development of full-blown disease. Therefore genetically engineered mouse models may be expected to provide further understanding of the disease mechanisms and time course. Another major advantage of genetic mouse models is their usefulness for compound screening in drug development, since rodents are much easier for preclinical drug screening from both economical and ethical viewpoints. For these reasons, genetic mouse models may provide an opportunity to identify novel therapeutic strategies that are directly linked to the mechanisms of psychological disorders.

Many investigators have considered the possibility that brain function and behavior are modulated by a combination of several genetic and environmental factors, and the concept of "pathway" is more likely to mimic the mechanisms than the effect of a single gene product. Therefore it is necessary to co-transfer more than one gene, such as by the methods of in utero electroporation.[28,78,79] This will make it possible to evaluate the synergistic influence or epistatic effect of multiple genetic factors, as well as to test how defects in neuronal network formation in early development lead to the behavioral abnormalities in adulthood.

In summary, we tried to establish a series of abnormalities in genetically engineered mice models based on the neurodevelopmental hypotheses of schizophrenia. It seems clear that the multiple similarities of genetic mouse model with schizophrenic patients indicate the potential for further understanding the pathogenesis of schizophrenia. Generation of these genetic mouse models should shed light on the etiology of schizophrenia and lead to more effective therapies in the future.

Acknowledgements This study was supported by Grants-in-Aid for Scientific Research (A) (22248033), Scientific Research (B) (20390073) (21390045), and Exploratory Research from the JSPS (19659017) (22659213) by the "Academic Frontier" Project for Private Universities (2007—2011) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT); by Regional Joint Research Program supported by Grants to Private Universities to Cover Current Expenses from MEXT, by Research on Regulatory Science of Pharmaceuticals and Medical Devices from the Ministry of Health and Labour and Welfare (MHLW); by Research on Risk of Chemical Substances, Health and Labour Science Research Grants supported by MHLW and by joint research project under the Japan–Korea basic scientific cooperation program by Japan Society for the Promotion of Science (JSPS). We thank Dr. Ping Lu for revising the manuscript.

REFERENCES

1) Cardno A. G., Marshall E. J., Coid B., Macdonald A. M., Ribchester T. R., Davies N. J., Venturi P., Jones L. A., Lewis S. W., Sham P. C., Gottesman I. I., Farmer A. E., McGuffin P., Reveley A. M., Murray R. M., *Arch. Gen. Psychiatry*, **56**, 162—168 (1999).
2) Singh S. M., McDonald P., Murphy B., O'Reilly R., *Clin. Genet.*, **65**, 435—440 (2004).
3) Stahl S. M., "Essential Psychopharmacology," Cambrige University Press, 2000.
4) Chen J., Lipska B. K., Weinberger D. R., *Biol. Psychiatry*, **59**, 1180—1188 (2006).
5) Jones E. G., *Ciba Found. Symp.*, **193**, 277—295 (1995).
6) Vicente A. M., Macciardi F., Verga M., Bassett A. S., Honer W. G., Bean G., Kennedy J. L., *Mol. Psychiatry*, **2**, 65—69 (1997).
7) Lipska B. K., Weinberger D. R., *Neurotox. Res.*, **4**, 469—475 (2002).
8) Arnold S. E., Talbot K., Hahn C. G., *Prog. Brain Res.*, **147**, 319—345 (2005).
9) Cardno A. G., Gottesman I. I., *Am. J. Med. Genet.*, **97**, 12—17 (2000).
10) Kohn Y., Lerer B., *Isr. J. Psychiatry Relat. Sci.*, **39**, 340—351 (2002).
11) Sullivan P. F., Kendler K. S., Neale M. C., *Arch. Gen. Psychiatry*, **60**, 1187—1192 (2003).
12) McGuffin P., Tandon K., Corsico A., *Curr. Psychiatry Rep.*, **5**, 121—127 (2003).
13) Gottesman I. I., Shields J., *Proc. Natl. Acad. Sci. U.S.A.*, **58**, 199—205 (1967).
14) Kozlovsky N., Belmaker R. H., Agam G., *Eur. Neuropsychopharmacol.*, **12**, 13—25 (2002).
15) McCarley R. W., Wible C. G., Frumin M., Hirayasu Y., Levitt J. J., Fischer I. A., Shenton M. E., *Biol. Psychiatry*, **45**, 1099—1119 (1999).
16) Shenton M. E., Dickey C. C., Frumin M., McCarley R. W., *Schizophr. Res.*, **49**, 1—52 (2001).
17) Gourion D., Gourevitch R., Leprovost J. B., Olié H., Lôo J. P., Krebs M. O., *Encephale*, **30**, 109—118 (2004).
18) Harrison P. J., *Brain*, **122**, 593—624 (1999).

19) Schurov I. L., Handford E. J., Brandon N. J., Whiting P. J., *Mol. Psychiatry*, **9**, 1100—1110 (2004).
20) Chubb J. E., Bradshaw N. J., Soares D. C., Porteous D. J., Millar J. K., *Mol. Psychiatry*, **13**, 36—64 (2008).
21) Brandon N. J., Millar J. K., Korth C., Sive H., Singh K. K., Sawa A., *J. Neurosci.*, **29**, 12768—12775 (2009).
22) Hikida T., Jaaro-Peled H., Seshadri S., Oishi K., Hookway C., Kong S., Wu D., Xue R., Andradé M., Tankou S., Mori S., Gallagher M., Ishizuka K., Pletnikov M., Kida S., Sawa A., *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 14501—14506 (2007).
23) Pletnikov M. V., Ayhan Y., Nikolskaia O., Xu Y., Ovanesov M. V., Huang H., Mori S., Moran T. H., Ross C. A., *Mol. Psychiatry*, **13**, 173—186 (2008).
24) Shen S., Lang B., Nakamoto C., Zhang F., Pu J., Kuan S. L., Chatzi C., He S., Mackie I., Brandon N. J., Marquis K. L., Day M., Hurko O., McCaig C. D., Riedel G., St Clair D., *J. Neurosci.*, **28**, 10893—10904 (2008).
25) Li W., Zhou Y., Jentsch J. D., Brown R. A., Tian X., Ehninger D., Hennah W., Peltonen L., Lönnqvist J., Huttunen M. O., Kaprio J., Trachtenberg J. T., Silva A. J., Cannon T. D., *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 18280—18285 (2007).
26) Kvajo M., McKellar H., Arguello P. A., Drew L. J., Moore H., MacDermott A. B., Karayiorgou M., Gogos J. A., *Proc. Natl. Acad. Sci. U.S.A.*, **105**, 7076—7081 (2008).
27) Mao Y., Ge X., Frank C. L., Madison J. M., Koehler A. N., Doud M. K., Tassa C., Berry E. M., Soda T., Singh K. K., Biechele T., Petryshen T. L., Moon R. T., Haggarty S. J., Tsai L. H., *Cell*, **136**, 1017—1031 (2009).
28) Niwa M., Kamiya A., Murai R., Kubo K., Gruber A. J., Tomita K., Lu L., Tomisato S., Jaaro-Peled H., Seshadri S., Hiyama H., Huang B., Kohda K., Noda Y., O'Donnell P., Nakajima K., Sawa A., Nabeshima T., *Neuron*, **65**, 480—489 (2010).
29) Stefansson H., Sigurdsson E., Steinthorsdottir V., Bjornsdottir S., Sigmundsson T., Ghosh S., Brynjolfsson J., Gunnarsdottir S., Ivarsson O., Chou T. T., Hjaltason O., Birgisdottir B., Jonsson H., Gudnadottir V. G., Gudmundsdottir E., Bjornsson A., Ingvarsson B., Ingason A., Sigfusson S., Hardardottir H., Harvey R. P., Lai D., Zhou M., Brunner D., Mutel V., Gonzalo A., Lemke G., Sainz J., Johannesson G., Andresson T., Gudbjartsson D., Manolescu A., Frigge M. L., Gurney M. E., Kong A., Gulcher J. R., Petursson H., Stefansson K., *Am. J. Hum. Genet.*, **71**, 877—892 (2002).
30) Stefansson H., Sarginson J., Kong A., Yates P., Steinthorsdottir V., Gudfinnsson E., Gunnarsdottir S., Walker N., Petursson H., Crombie C., Ingason A., Gulcher J. R., Stefansson K., St Clair D., *Am. J. Hum. Genet.*, **72**, 83—87 (2003).
31) Li T., Stefansson H., Gudfinnsson E., Cai G., Liu X., Murray R. M., Steinthorsdottir V., Januel D., Gudnadottir V. G., Petursson H., Ingason A., Gulcher J. R., Stefansson K., Collier D. A., *Mol. Psychiatry*, **9**, 698—704 (2004).
32) Stefansson H., Steinthorsdottir V., Thorgeirsson T. E., Gulcher J. R., Stefansson K., *Ann. Med.*, **36**, 62—71 (2004).
33) Hashimoto R., Straub R. E., Weickert C. S., Hyde T. M., Kleinman J. E., Weinberger D. R., *Mol. Psychiatry*, **9**, 299—307 (2004).
34) Petryshen T. L., Middleton F. A., Kirby A., Aldinger K. A., Purcell S., Tahl A. R., Morley C. P., McGann L., Gentile K. L., Rockwell G. N., Medeiros H. M., Carvalho C., Macedo A., Dourado A., Valente J., Ferreira C. P., Patterson N. J., Azevedo M. H., Daly M. J., Pato C. N., Pato M. T., Sklar P., *Mol. Psychiatry*, **10**, 366—374 (2005).
35) Gu Z., Jiang Q., Fu A. K., Ip N. Y., Yan Z., *J. Neurosci.*, **25**, 4974—4984 (2005).
36) Brinkmann B. G., Agarwal A., Sereda M. W., Garratt A. N., Müller T., Wende H., Stassart R. M., Nawaz S., Humml C., Velanac V., Radyushkin K., Goebbels S., Fischer T. M., Franklin R. J., Lai C., Ehrenreich H., Birchmeier C., Schwab M. H., Nave K. A., *Neuron*, **59**, 581—595 (2008).
37) Barros C. S., Calabrese B., Chamero P., Roberts A. J., Korzus E., Lloyd K., Stowers L., Mayford M., Halpain S., Müller U., *Proc. Natl. Acad. Sci. U.S.A.*, **106**, 4507—4512 (2009).
38) Fisahn A., Neddens J., Yan L., Buonanno A., *Cereb. Cortex*, **19**, 612—618 (2009).
39) Flames N., Long J. E., Garratt A. N., Fischer T. M., Gassmann M., Birchmeier C., Lai C., Rubenstein J. L., Marín O., *Neuron*, **44**, 251—261 (2004).
40) Schwab S. G., Knapp M., Mondabon S., Hallmayer J., Borrmann-Hassenbach M., Albus M., Lerer B., Rietschel M., Trixler M., Maier W., Wildenauer D. B., Rietschel M., Lerer B., Maier W., Wildenauer D. B., *Am. J. Hum. Genet.*, **72**, 185—190 (2003).
41) Straub R. E., Jiang Y., MacLean C. J., Ma Y., Webb B. T., Myakishev M. V., Harris-Kerr C., Wormley B., Sadek H., Kadambi B., Cesare A. J., Gibberman A., Wang X., O'Neill F. A., Walsh D., Kendler K. S., *Am. J. Hum. Genet.*, **71**, 337—348 (2002).
42) Talbot K., Eidem W. L., Tinsley C. L., Benson M. A., Thompson E. W., Smith R. J., Hahn C. G., Siegel S. J., Trojanowski J. Q., Gur R. E., Blake D. J., Arnold S. E., *J. Clin. Invest.*, **113**, 1353—1363 (2004).
43) Li W., Zhang Q., Oiso N., Novak E. K., Gautam R., O'Brien E. P., Tinsley C. L., Blake D. J., Spritz R. A., Copeland N. G., Jenkins N. A., Amato D., Roe B. A., Starcevic M., Dell'Angelica E. C., Elliott R. W., Mishra V., Kingsmore S. F., Paylor R. E., Swank R. T., *Nat. Genet.*, **35**, 84—89 (2003).
44) Chen X. W., Feng Y. Q., Hao C. J., Guo X. L., He X., Zhou Z. Y., Guo N., Huang H. P., Xiong W., Zheng H., Zuo P. L., Zhang C. X., Li W., Zhou Z., *J. Cell Biol.*, **181**, 791—801 (2008).
45) Feng Y. Q., Zhou Z. Y., He X., Wang H., Guo X. L., Hao C. J., Guo Y., Zhen X. C., Li W., *Schizophr. Res.*, **106**, 218—228 (2008).
46) Green E., Craddock N., *Curr. Psychiatry Rep.*, **5**, 469—476 (2003).
47) Knable M. B., Barci B. M., Webster M. J., Meador-Woodruff J., Torrey E. F., *Mol. Psychiatry*, **9**, 609—620 (2004).
48) Weickert C. S., Ligons D. L., Romanczyk T., Ungaro G., Hyde T. M., Herman M. M., Weinberger D. R., Kleinman J. E., *Mol. Psychiatry*, **10**, 637—650 (2005).
49) Kernie S. G., Liebl D. J., Parada L. F., *EMBO J.*, **19**, 1290—1300 (2000).
50) Lyons W. E., Mamounas L. A., Ricaurte G. A., Coppola V., Reid S. W., Bora S. H., Wihler C., Koliatsos V. E., Tessarollo L., *Proc. Natl. Acad. Sci. U.S.A.*, **96**, 15239—15244 (1999).
51) Gorski J. A., Balogh S. A., Wehner J. M., Jones K. R., *Neuroscience*, **121**, 341—354 (2003).
52) Akbarian S., Rios M., Liu R. J., Gold S. J., Fong H. F., Zeiler S., Coppola V., Tessarollo L., Jones K. R., Nestler E. J., Aghajanian G. K., Jaenisch R., *J. Neurosci.*, **22**, 4153—4162 (2002).
53) Chourbaji S., Hellweg R., Brandis D., Zörner B., Zacher C., Lang U. E., Henn F. A., Hörtnagl H., Gass P., *Brain Res. Mol. Brain Res.*, **121**, 28—36 (2004).
54) Monteggia L. M., Barrot M., Powell C. M., Berton O., Galanis V., Gemelli T., Meuth S., Nagy A., Greene R. W., Nestler E. J., *Proc. Natl. Acad. Sci. U.S.A.*, **101**, 10827—10832 (2004).
55) Akahane A., Kunugi H., Tanaka H., Nanko S., *Schizophr. Res.*, **58**, 37—41 (2002).
56) Egan M. F., Weinberger D. R., Lu B., *Am. J. Psychiatry*, **160**, 1242 (2003).
57) Costa E., Davis J., Pesold C., Tueting P., Guidotti A., *Curr. Opin. Pharmacol.*, **2**, 56—62 (2002).
58) Guidotti A., Auta J., Davis J. M., Di-Giorgi-Gerevini V., Dwivedi Y., Grayson D. R., Impagnatiello F., Pandey G., Pesold C., Sharma R., Uzunov D., Costa E., *Arch. Gen. Psychiatry*, **57**, 1061—1069 (2000).
59) Impagnatiello F., Guidotti A. R., Pesold C., Dwivedi Y., Caruncho H., Pisu M. G., Uzunov D. P., Smalheiser N. R., Davis J. M., Pandey G. N., Pappas G. D., Tueting P., Sharma R. P., Costa E., *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 15718—15723 (1998).
60) Duncan G. E., Moy S. S., Perez A., Eddy D. M., Zinzow W. M., Lieberman J. A., Snouwaert J. N., Koller B. H., *Behav. Brain Res.*, **153**, 507—519 (2004).
61) Fradley R. L., O'Meara G. F., Newman R. J., Andrieux A., Job D., Reynolds D. S., *Behav. Brain Res.*, **163**, 257—264 (2005).
62) Mohn A. R., Gainetdinov R. R., Caron M. G., Koller B. H., *Cell*, **98**, 427—436 (1999).
63) Meador-Woodruff J. H., Healy D. J., *Brain Res. Brain Res. Rev.*, **31**, 288—294 (2000).
64) Sokolov B. P., *J. Neurochem.*, **71**, 2454—2464 (1998).
65) Toyoda H., Takahata R., Inayama Y., Sakai J., Matsumura H., Yoneda H., Asaba H., Sakai T., *Neurochem. Res.*, **22**, 249—252 (1997).
66) Heresco-Levy U., Ermilov M., Lichtenberg P., Bar G., Javitt D. C., *Biol. Psychiatry*, **55**, 165—171 (2004).
67) Tsai G., Coyle J. T., *Annu. Rev. Pharmacol. Toxicol.*, **42**, 165—179 (2002).
68) Belforte J. E., Zsiros V., Sklar E. R., Jiang Z., Yu G., Li Y., Quinlan E. M., Nakazawa K., *Nat. Neurosci.*, **13**, 76—83 (2010).
69) Koizumi H., Yamaguchi N., Hattori M., Ishikawa T. O., Aoki J., Taketo

M. M., Inoue K., Arai H., *J. Biol. Chem.*, **278**, 12489—12494 (2003).
70) Ohshima T., Ward J. M., Huh C. G., Longenecker G. Veeranna, Pant H. C., Brady R. O., Martin L. J., Kulkarni A. B., *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 11173—11178 (1996).
71) Paylor R., Zhao Y., Libbey M., Westphal H., Crawley J. N., *Physiol. Behav.*, **73**, 781—792 (2001).
72) Wood G. K., Tomasiewicz H., Rutishauser U., Magnuson T., Quirion R., Rochford J., Srivastava L. K., *Neuroreport*, **9**, 461—466 (1998).
73) Harrison P. J., Weinberger D. R., *Mol. Psychiatry*, **10**, 40—68 (2005).
74) Sinor A. D., Lillien L., *J. Neurosci.*, **24**, 8531—8541 (2004).
75) Liu Y., Ford B. D., Mann M. A., Fischbach G. D., *Dev. Biol.*, **283**, 437—445 (2005).
76) Duan X., Chang J. H., Ge S., Faulkner R. L., Kim J. Y., Kitabatake Y., Liu X. B., Yang C. H., Jordan J. D., Ma D. K., Liu C. Y., Ganesan S., Cheng H. J., Ming G. L., Lu B., Song H., *Cell*, **130**, 1146—1158 (2007).
77) Le Strat Y., Ramoz N., Gorwood P., *Curr. Mol. Med.*, **9**, 506—518 (2009).
78) Kamiya A., Tan P. L., Kubo K., Engelhard C., Ishizuka K., Kubo A., Tsukita S., Pulver A. E., Nakajima K., Cascella N. G., Katsanis N., Sawa A., *Arch. Gen. Psychiatry*, **65**, 996—1006 (2008).
79) Young-Pearse T. L., Bai J., Chang R., Zheng J. B., LoTurco J. J., Selkoe D. J., *J. Neurosci.*, **27**, 14459—14469 (2007).

No.24
Neuropsychopharmacology (2012) 37, 2522–2530

www.neuropsychopharmacology.org

Cortico-Subcortical Neuromodulation Involved in the Amelioration of Prepulse Inhibition Deficits in Dopamine Transporter Knockout Mice

Yosefu Arime[1,2], Yoshiyuki Kasahara[1], F Scott Hall[3], George R Uhl[3] and Ichiro Sora*[,1]

[1]Department of Biological Psychiatry, Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Department of Biological Psychiatry and Neuroscience, Dokkyo Medical University School of Medicine, Mibu, Japan; [3]Molecular Neurobiology Branch, National Institute on Drug Abuse, Division of Intramural Research, NIH/DHSS, Baltimore, MD, USA

Prepulse inhibition (PPI) deficits are among the most reproducible phenotypic markers found in schizophrenic patients. We recently reported that nisoxetine, a selective norepinephrine transporter (NET) inhibitor, reversed the PPI deficits that have been identified in dopamine transporter (DAT) knockout (KO) mice. However, the mechanisms underlying nisoxetine-induced PPI recovery in DAT KO mice were unclear in previous experiments. To clarify these mechanisms, PPI was tested after microinjections of nisoxetine into the medial prefrontal cortex (mPFc) or nucleus accumbens (NAc) in wildtype (WT) and DAT KO mice. c-Fos immunohistochemistry provided an indicator of neural activation. Multiple-fluorescent-labeling procedures and the retrograde tracer fluorogold were employed to identify nisoxetine-activated neurons and circuits. Systemic nisoxetine activated the mPFc, the NAc shell, the basolateral amygdala, and the subiculum. Infusions of nisoxetine into the mPFc reversed PPI deficits in DAT KO mice, but produced no changes in WT mice, while infusion of nisoxetine into the NAc had no effect on PPI in both WT and DAT KO mice. Experiments using multiple-fluorescent labeling/fluorogold revealed that nisoxetine activates presumed glutamatergic pyramidal cells that project from the mPFc to the NAc. Activated glutamatergic projections from the mPFc to the NAc appear to have substantial roles in the ability of a NET inhibitor to normalize PPI deficits in DAT KO. Thus, this data suggest that selective NET inhibitors such as nisoxetine might improve information processing deficits in schizophrenia *via* regulation of cortico-subcortical neuromodulation.
Neuropsychopharmacology (2012) **37,** 2522–2530; doi:10.1038/npp.2012.114; published online 11 July 2012

Keywords: prepulse inhibition; norepinephrine reuptake inhibitor; medial prefrontal cortex; neural circuits; mouse

INTRODUCTION

Cognitive dysfunction, including problems in attention, learning, memory, and executive functions, are core symptoms of schizophrenia that are strongly associated with functional impairments in daily life (Mueser and McGurk, 2004). Most antipsychotic medications display only modest, if any, enhancement of cognitive performance in schizophrenics (Tamminga, 2006; Goldberg *et al*, 2007; Keefe *et al*, 2007). Medications that reverse aspects of the cognitive dysfunction in schizophrenic patients are thus urgently needed.

Hyperdopaminergic tone and hypofunction of glutamatergic signaling have both been suggested to contribute to cognitive dysfunction in schizophrenics and to underlie behavioral and cognitive deficits in rodent models of schizophrenia (Jentsch and Roth, 1999; Iversen and Iversen, 2007). Dopamine transporter knockout (DAT KO) mice exhibit increased extracellular dopamine levels in the striatum and nucleus accumbens (NAc), but display normal medial prefrontal cortical (mPFc) dopamine levels (Shen *et al*, 2004). The DAT KO mice display deficits in prepulse inhibition (PPI), a model of sensorimotor gating (Ralph *et al*, 2001; Yamashita *et al*, 2006; Ishii *et al*, 2010). PPI is the suppression of the startle response that occurs when a startle-eliciting stimulus is preceded by a brief low-intensity stimulus (the prepulse). As this response can be reliably elicited in rodents and humans, this model for identifying sensorimotor gating deficits has served as one of the few validated animal models of a clinical feature of abnormalities in sensorimotor gating impairments in schizophrenics (although PPI deficits can also be found in some other psychiatric disorders) (Braff *et al*, 2001; Geyer, 2006; Swerdlow *et al*, 2006). PPI has neuroanatomical substrates that include the mPFc, basolateral amygdala (BLA), hippocampus, and NAc, and neurochemical substrates that include dopamine, glutamate, and GABA (Swerdlow *et al*, 2001; Schmajuk and Larrauri, 2005). We previously

*Correspondence: Dr I Sora, Department of Biological Psychiatry, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Sendai, 980-8574, Japan, Tel: +81 22 717 8593, Fax: +81 22 717 7809, E-mail: sora@med.tohoku.ac.jp
Received 23 August 2011; revised 4 June 2012; accepted 6 June 2012

reported that systemic treatment with a selective norepinephrine reuptake inhibitor (NRI), nisoxetine, normalized PPI deficits observed in DAT KO mice (Yamashita *et al*, 2006). However, the brain circuits at which nisoxetine might act to mediate recovery of PPI in DAT KO mice have yet to be determined (Schroeter *et al*, 2000; Gehlert *et al*, 2008). We now report results that elucidate the neural circuits that underlie PPI recovery in DAT KO mice by mapping the changes in neurons that express c-Fos (Sumner *et al*, 2004) after systemic nisoxetine administration, in combination with nisoxetine microinjection, specific neural markers and fluorogold.

MATERIALS AND METHODS

Animals

DAT KO mice and wildtype (WT) littermates (Sora *et al*, 1998) were bred at the Animal Laboratory Institute of Tohoku University Graduate School of Medicine and maintained on a mixed C57BL/6J-129Sv genetic background by crossing between heterozygous mice. Mice were weaned at 4 weeks of age and housed socially (segregated by sex), in a temperature- (22.0–24.0 °C), humidity- (45–65%) and light-controlled room (light on 0800–2000 hours). Food and water were available *ad libitum*. Male and female mice from 8–20 weeks old of each genotype were used in each experiment in equal proportions. All experiments were performed in accordance with the Guidelines for Care of Laboratory Animals of Tohoku University Graduate School of Medicine and conformed to all Japanese federal rules and guidelines.

Drug Administration

For immunohistochemical studies, nisoxetine hydrochloride (Sigma-Aldrich) was dissolved in saline (10 mg/kg), and administered intraperitoneally (i.p.) in a volume of 10 ml/kg. For intracerebral microinjection studies, nisoxetine (2 and 8 μg/side) was dissolved in Ringer's solution (Na^+ 145 mM, K^+ 3 mM, Ca^{2+} 1.26 mM, and Mg^{2+} 1 mM).

Immunohistochemical Analysis of c-Fos Expression

WT and DAT KO mice were used to identify the regions of the brain activated by nisoxetine. Mice were placed in a temperature- (23.0 °C) and light-controlled (light on 0800–2000 hours) chamber on the day before the experiment. On the following day, 2 h after nisoxetine administration (10 mg/kg, i.p.), mice were deeply anesthetized with nembutal and transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). This interval (2 h) was determined from the following: our previous work reported that amelioration of PPI deficits in DAT KO mice was observed 30 min after systemic nisoxetine treatment (Yamashita *et al*, 2006), and the period of peak production for c-Fos protein was between 90 and 120 min after treatment (Bisler *et al*, 2002). Brains were removed and post-fixed in the same fixative, transferred to 15% sucrose in 0.1 M PB, and then immersed in 30% sucrose in 0.1 M PB for cryoprotection. Brains were sectioned on a freezing microtome and serial coronal sections (14 μm) were incubated in PBS with 10% normal horse serum and 0.5% Triton X-100, and then incubated overnight at 4 °C with rabbit anti-c-Fos polyclonal antibody (1:1000, sc-253, Santa Cruz Biotechnology). Sections were rinsed with PBS and incubated with goat Alexa 488-conjugated anti-rabbit IgG (1:300, A11034, Molecular Probes) at 4 °C for 1 h. Sections were transferred to slides, rinsed with PBS, and imaged using a fluorescence microscope (Leica DMRXA).

Medial prefrontal cortex (mPFc), NAc shell and core, BLA, and dorsal and ventral hippocampus (dHPC and vHPC) were located according to the coordinates of the Paxinos mouse brain atlas (Paxinos, 2001). Subjects were numbered so that the investigator was blind to the groups during analysis. The numbers of c-Fos-positive cells in each region were quantified manually within a $744 \times 555\ \mu m^2$ grid placed over each area from each of four sections using ImageJ. Statistical analyses used the SPSS statistical package (SPSS 11.5J for Windows, SPSS, Tokyo, Japan).

Surgery

WT and DAT KO mice were anesthetized using Avertin (20 mg/ml), injected i.p., at an initial dose of 0.1 ml/10 gm body weight, and bilaterally implanted with guide cannulae targeting the mPFc (anterior +1.8 mm, lateral +0.5 mm, ventral −1.7 mm from bregma) or NAc shell (anterior +1.2 mm, lateral +0.6 mm, ventral −4.7 mm from bregma) according to the atlas of Paxinos (Paxinos, 2001). Mice were allowed to recover for at least 5 days before PPI testing and/or fluorogold infusions were performed.

PPI Testing in DAT KO Mice

PPI testing was conducted using previously reported methods (Geyer and Dulawa, 2003). Mice were tested in startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), consisting of a nonrestrictive Plexiglas cylinder mounted on a frame inside a lighted, ventilated, and sound-proof chamber ($35 \times 35 \times 47.5\ cm^3$). Movement was detected by a piezoelectric accelerometer. Electrical signals were digitized and stored by a computer. A total of 65 readings were recorded at 1 ms intervals beginning at the stimulus onset. Average amplitude over this time was used as the measure of startle. Experimental sessions consisted of a 5 min acclimatization period with 65 dB background noise followed by startle trials, which were of five different types: no stimulus (nostim), consisting only of background noise (65 dB); startle pulse alone, 40 ms duration at 120 dB (p120); and three prepulse + pulse conditions, 20 ms duration prepulse at 68 dB (pp3), 71 dB (pp6), or 77 dB (pp12), followed by a 40 ms duration startle stimulus at 120 dB after a 100 ms interstimulus interval. The duration of the interstimulus interval was measured between the onset of the prepulse and the onset of the pulse (onset to onset). Test sessions began and ended with 12 presentations of the p120 trial, followed by 10 presentations of the nostim trial, and pp3, pp6, and pp12 prepulse trials given in pseudorandom order, with an intertrial interval of 8–23 s (mean 15 s). PPI was calculated as the percentage of the startle magnitude at each prepulse intensity using the following equation: % PPI = 100−((startle response for

prepulse + pulse (pp3, pp6, or pp12))/(startle response for pulse alone (p120)) × 100). In the microinjection study, nisoxetine solution (2 and 8 μg/side or vehicle (Ringer's solution) was microinjected bilaterally into freely moving mice using a microsyringe pump (0.25 μl/min for 2 min). After the microinjection, the cannulae were left in place for 1 min to allow diffusion of the drug. Mice were then placed into the startle chamber for PPI testing. After the microinjection study, the brains were removed, sectioned at 100 μm thickness using a vibratome, and stained with cresyl violet for localization of cannula placements.

Double Immunostaining for c-Fos and Neural Markers

Brain sections obtained from naïve WT ($n=4$; $n=2$ male and $n=2$ female) and DAT KO mice ($n=4$; $n=2$ male and $n=2$ female) were boiled in 10 mM citrate solution for 5 min. Sections were washed with PBS, incubated in PBS with 10% normal horse serum and 0.5 % Triton X-100, and then incubated overnight at 4 °C with rabbit anti-c-Fos polyclonal antibody (1:1000, sc-253, Santa Cruz Biotechnology), anti-GAD67 monoclonal antibody (1:500, MAB5406, Chemicon), anti-phosphate-activated glutaminase (PAG) monoclonal antibody (1:500, 5 mg/ml; a gift from Takeshi Kaneko, Kyoto University) or anti-tyrosine hydroxylase (TH) monoclonal antibody (1:1000, MAB318, Chemicon) as markers for activated neurons, GABAergic, glutamatergic or dopaminergic neurons, respectively. Sections were washed with PBS, incubated with biotinylated anti-mouse IgG (5 μg/ml, BA-2000, Vector Laboratories) at 4 °C for 2 h, incubated with goat Alexa 594-conjugated anti-rabbit IgG (1:300, A11012, Molecular Probes) and Alexa 488-conjugated streptavidin (1:300, S11223, Molecular Probes) at 4 °C for 1 h, rinsed with PBS and imaged using a fluorescence microscope (Keyence, BZ-9000). Quantitative analysis of c-Fos and PAG colocalization was calculated as the percentage of colocalized neurons: Colocalized neurons (%) = (Number of c-Fos-positive and PAG-positive cells)/(Number of c-Fos-positive cells) × 100.

Fluorogold Infusion and Triple Fluorescence Imaging

WT and DAT KO mice implanted with guide cannulae in the NAc, as described above, were used for fluorogold-retrograde labeling and immunostaining to detect multiple neuronal markers. Fluorogold (80014, Biotium) was dissolved in saline at a concentration of 4% and injected bilaterally (0.5 μl/side) into the NAc. Sections were prepared as described above, up to the secondary antibody step, but were then incubated with goat Alexa 555-conjugated anti-rabbit IgG (1:200, A21429, Molecular Probes) and Alexa 633-conjugated streptavidin (1:200, S21375, Molecular Probes) at 4 °C for 1 h, rinsed with PBS, and imaged by fluorescence microscopy (Keyence, BZ-9000). Quantitative analysis of colocalization of c-Fos, neuronal markers, and fluorogold was calculated as the percentage of colocalized neurons: Colocalized neurons (%) in the mPFc = (Number of triple-labeled cells for c-Fos, PAG, and fluorogold)/(Number of c-Fos-positive cells) × 100, colocalized neurons (%) in the VTA = (Number of triple-labeled cells for c-Fos, TH, and fluorogold)/(Number of c-Fos-positive cells) × 100.

Statistical Analysis

For c-Fos immunohistochemistry, cell counts were analyzed by analysis of variance (ANOVA), with brain region, genotype, and drug as between-subjects factors, followed by the Tukey HSD or Bonferroni *post hoc* comparisons. PPI data in microinjection studies were analyzed by ANOVA with drug treatment and genotype as between-subjects factors and prepulse intensities as a within-subjects factor followed by the Bonferroni *post hoc* comparisons. The alpha level <5% ($p<0.05$) was considered as significant difference. All data are presented as means ± SEM.

RESULTS

Effects of Nisoxetine on c-Fos Induction

c-Fos immunoreactivity was examined in WT and DAT KO mice to identify the brain regions that were activated by nisoxetine. Systemic nisoxetine (10 mg/kg), which normalized PPI defects in DAT KO mice (see below), increased the number of c-Fos-positive nuclei in several brain regions (Figure 1). ANOVA revealed that there was no significant main effect of genotype on the number of c-Fos-positive nuclei, there were significant main effect of drug ($F(1,404) = 91.286$, $p<0.001$) and brain region ($F(5,404)=14.286$, $p<0.001$), there was a significant drug × brain region interaction ($F(5,404)=11.726$, $p<0.001$), and there was no significant brain region × drug × genotype interaction. *Post hoc* analysis revealed a significant induction of c-Fos by nisoxetine in the mPFc ($F(1,404)=70.003$, $p<0.001$), NAc shell ($F(1,404)=32.114$, $p<0.001$), BLA ($F(1,404)=48.684$, $p<0.001$), and vHPC ($F(1,404)=6.009$, $p<0.05$). By contrast, there were no changes in the number of c-Fos-positive nuclei in the NAc core and dHPC.

Effects of Nisoxetine Microinjection on PPI

There were no effects of surgery on PPI in WT or DAT KO mice; neither PPI nor startle response differed between

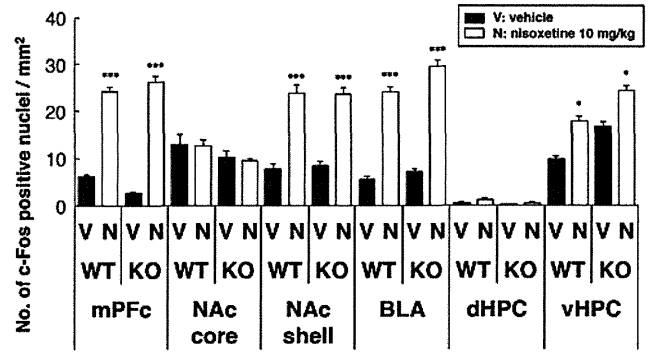


Figure 1 The effects of vehicle (V) or selective norepinephrine reuptake inhibitor, nisoxetine (N) 10 mg/kg, on the numbers of c-Fos-positive nuclei in WT ($n=4$–5; $n=2$–3 for male and $n=2$–3 for female per groups) and DAT KO mice ($n=4$–5; $n=2$–3 for male and $n=2$–3 for female per groups). Nisoxetine significantly increased the numbers of c-Fos-positive nuclei in the medial prefrontal cortex (mPFc), nucleus accumbens (NAc) shell, basolateral amygdala (BLA), and ventral hippocampus (vHPC) in both WT and DAT KO mice. Nisoxetine had no effect on c-Fos expression in the NAc core and dorsal hippocampus (dHPC). ***$p<0.001$, *$p<0.05$ compared with vehicle-treated mice.

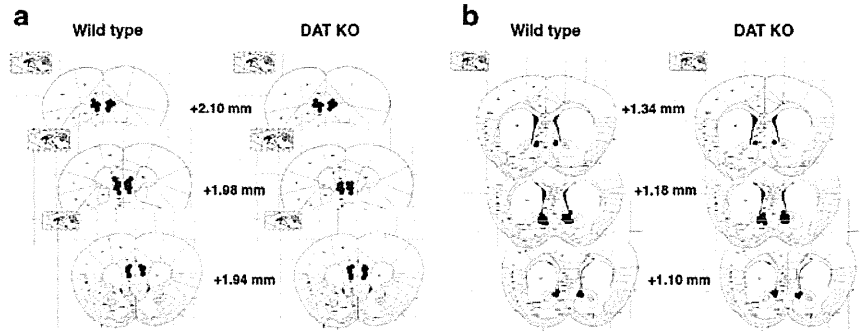


Figure 2 Schematic representation of the injection sites, mapped on drawings of mouse brain coronal sections (adapted from Paxinos, 2001). Each black circle in (a) medial prefrontal cortex (mPFc) and (b) nucleus accumbens (NAc) represents the approximate injection cannula placement.

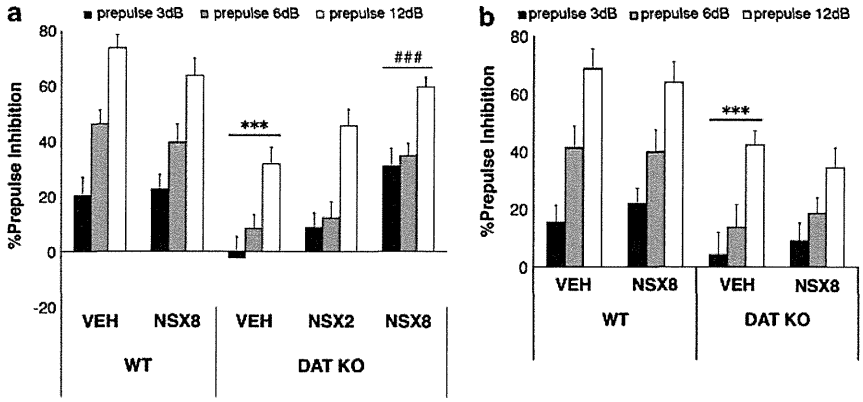


Figure 3 The effects of vehicle (VEH) or nisoxetine (NSX) microinjection on PPI in WT ($n = 11$–16 for medial prefrontal cortex (mPFc) infusion; $n = 6$–8 for male and $n = 5$–8 for female per groups; $n = 12$ for nucleus accumbens (NAc) infusion; $n = 6$ for male and $n = 6$ for female per groups) and DAT KO mice ($n = 12$–16 for mPFc infusion; $n = 6$–8 for male and $n = 6$–8 for female per groups; $n = 11$–12 for NAc infusion; $n = 5$–6 for male and $n = 6$ for female per groups). After administration of VEH into the mPFc (a) or NAc (b), DAT KO mice displayed significantly reduced PPI compared with WT VEH. mPFc infusions of nisoxetine (8 μg/side, NSX8) significantly increased PPI in DAT KO mice without affecting PPI in WT mice, while NAc infusions of nisoxetine (8 μg/side, NSX8) had no effect on PPI. % PPI values represent mean ± SEM. $^{***}p < 0.001$ compared with WT VEH; $^{\#\#\#}p < 0.01$ compared with KO VEH.

preoperative and postoperative assessments (data not shown). After intracerebral vehicle infusions into either the mPFC or NAc, PPI deficits were observed in DAT KO mice compared with WT mice (Figures 2, 3a and b). ANOVA revealed a significant main effect of genotype ($F(1, 61) = 13.0$, $p < 0.01$), drug ($F(2, 61) = 3.49$), and prepulse intensity ($F(2, 122) = 107.3$, $p < 0.001$) on PPI in mice with mPFC cannula. Intracerebral injection of nisoxetine into the mPFC reversed these deficits that were confirmed by a significant genotype × drug interaction in the ANOVA ($F(1, 61) = 12.7$, $p < 0.01$). *Post hoc* comparisons revealed that vehicle-treated DAT KO mice displayed PPI that was significantly reduced when compared with vehicle-treated WT mice ($F(1, 61) = 30.8$, $p < 0.001$). Nisoxetine 8 μg/side in the mPFc reversed the PPI deficits displayed by DAT KO mice compared with vehicle-treated DAT KO mice ($F(2, 61) = 9.72$, $p < 0.001$) (Figure 3a). Nisoxetine in the mPFC was without effect in WT mice. In contrast to the effects of nisoxetine in the mPFC, nisoxetine in the NAc did not significantly alter PPI in either WT or DAT KO mice (Figure 3b). ANOVA revealed a significant main effect of genotype ($F(1, 47) = 14.791$, $p < 0.001$), but there was no significant genotype × drug interaction.

Table 1 Effects of Intracerebral Injection on Acoustic Startle Reactivity

	Wild-type	DAT KO
Prefrontal cortex		
Vehicle	221.4 ± 37.5	139.4 ± 25.4
Nisoxetine 2 μg/side	ND	156.2 ± 29.1
Nisoxetine 8 μg/side	195.3 ± 36.9	164.1 ± 29.5
Nucleus accumbens		
Vehicle	161.6 ± 30.9	131.9 ± 31.3
Nisoxetine 8 μg/side	130.7 ± 21.8	144.6 ± 27.5

Abbreviations: DAT KO, dopamine transporter knockout mice; ND, not determined.
Each value represents the mean startle magnitude ± SEM.

The magnitude of the acoustic startle response was not affected by genotype or drug in either injection group (Table 1). In the ANOVA, there was no significant effect of genotype or drug, nor was there a significant genotype × drug interaction.

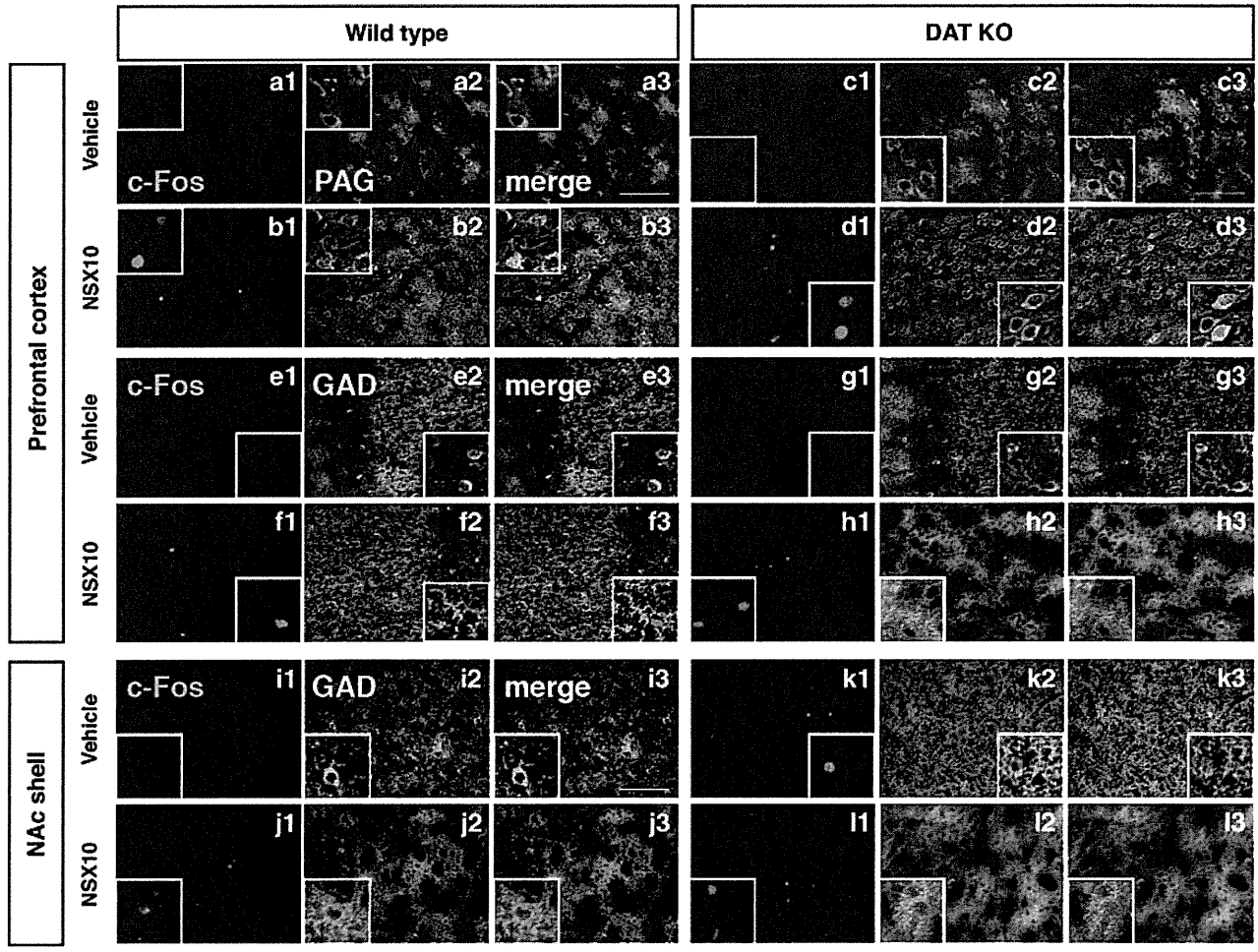


Figure 4 Representative micrographs of c-Fos (a1–l1, magenta), PAG (a2–d2, green), and GAD67 (e2–l2, green) immunoreactivity and overlay (a3–l3, merge) in WT ($n=4$; $n=2$ for male and $n=2$ for female) and DAT KO mice ($n=4$; $n=2$ for male and $n=2$ for female) treated with vehicle and nisoxetine (10 mg/kg, i.p.). Detailed quantitative data are shown in Table 2. PAG: glutamatergic neuronal marker, NAc: nucleus accumbens. Scale bars, 100 μm.

Double Immunostaining for c-Fos and Neuron Markers

To investigate the types of neurons activated by nisoxetine treatment, double immunostaining was employed for c-Fos and neuronal markers for glutamatergic (PAG) and GABAergic (GAD67) neurons. Systemic treatment with the selective norepinephrine transporter (NET) blocker nisoxetine activated PAG-positive neurons in the mPFc (Figure 4a–h). Table 2 shows that nearly all (WT mice: 94.2% and DAT KO mice: 87.4%) of the nisoxetine-induced c-Fos-positive cells in the mPFc express PAG. In addition to the glutamatergic neurons in these areas, nisoxetine activated GABAergic neurons in the NAc shell in both WT and DAT KO mice (Figure 4i–l).

Activated Neural Circuits by Nisoxetine Treatment

Retrograde labeling produced by fluorogold injection in the NAc was observed in neurons in the mPFc (Figure 5b and f) and VTA (Figure 5j and n) in both WT and DAT KO mice. Triple fluorescence immunostaining revealed the majority of nisoxetine-induced c-Fos expression in the mPFc was labeled for both PAG and fluorogold (WT mice: 84.0% and DAT KO mice: 90.9%; Figure 5d and h and Table 3). By contrast, no c-Fos expression was observed in both TH- and fluorogold-positive cells in the VTA (WT mice: 0% and DAT KO mice: 0%; Figure 5l and p).

Table 2 Proportion of Nisoxetine-Induced c-Fos+ that Express PAG in the mPFc

	Wild-type	DAT KO
Number of c-Fos+ (/mm^2)	16.8	17.4
Number of c-Fos+/PAG+ (/mm^2)	15.7	15.1
Colocalized neurons (%)	94.2	87.4

Abbreviations: c-Fos+, c-Fos-positive cells; PAG+, PAG-positive cells.

DISCUSSION

The present study found that (1) selective NET blockade by microinjection of nisoxetine into the mPFc, but not the NAc, ameliorated PPI deficits in DAT KO mice and

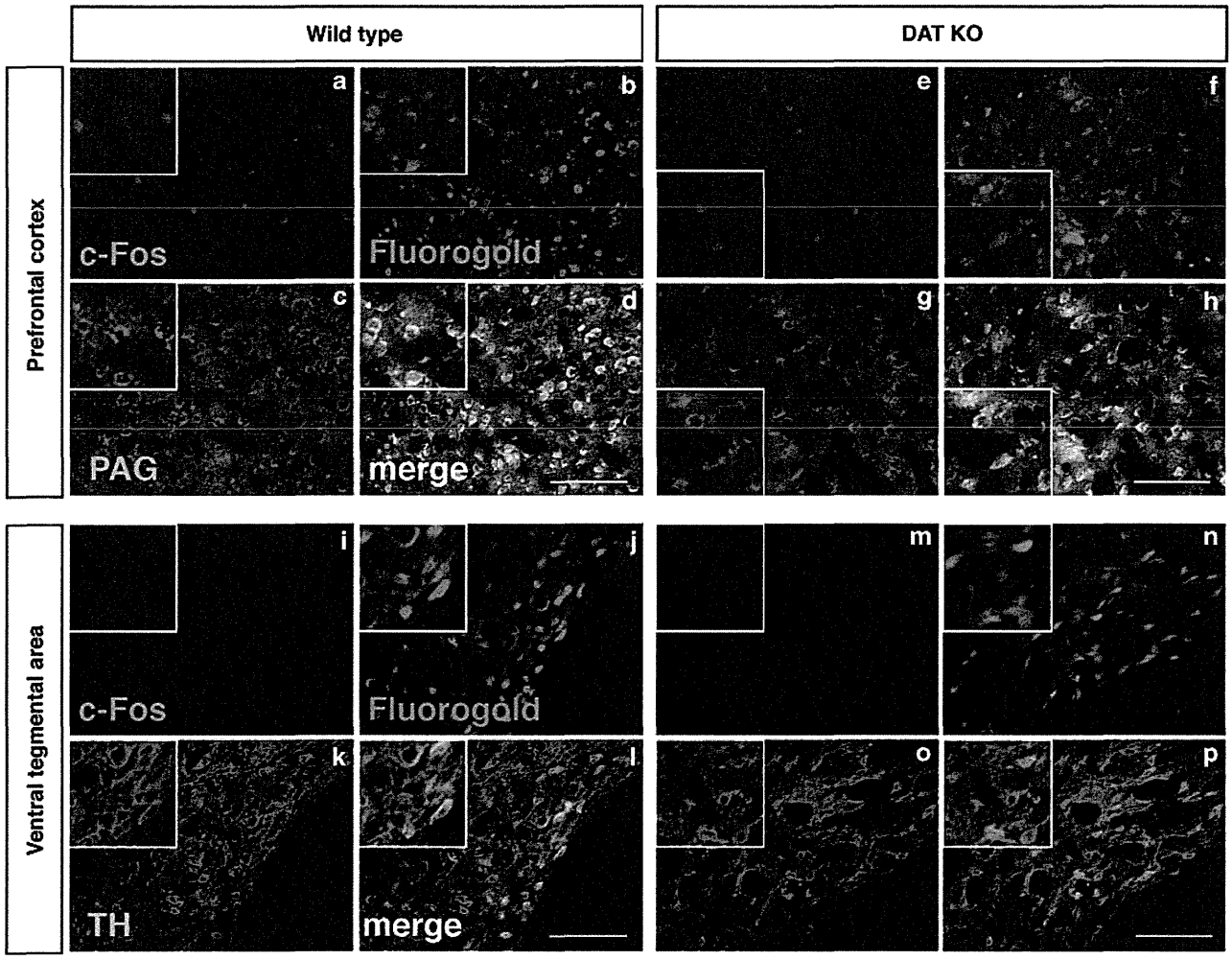


Figure 5 Triple fluorescence micrographs for c-Fos (blue; a, e, i, and m), PAG (magenta: c and g), TH (magenta; k and o), and fluorogold (green; b, f, j, and n) for WT and DAT KO mice treated with nisoxetine (10 mg/kg, i.p.) after fluorogold injection into the NAc. PAG (phosphate-activated glutaminase): glutamatergic neuronal marker and TH (tyrosine hydroxylase): dopaminergic marker. Scale bars, 100 μm.

Table 3 Proportion of Nisoxetine-Induced c-Fos+ that Colocalize PAG and Fluorogold in the mPFc

	Wild-type	DAT KO
Number of c-Fos+ (/mm^2)	28.7	30.2
Number of c-Fos+/PAG+/FG+ (/mm^2)	24.2	27.4
Colocalized neurons (%)	84.0	90.9

Abbreviations: c-Fos+, c-Fos-positive cells; FG+, fluorogold-labeled cells; PAG+, PAG positive cells.

(2) systemic nisoxetine activated glutamatergic pyramidal cells projecting to the NAc. This confirms the observation of nisoxetine-induced normalization of PPI deficits in DAT KO mice (Yamashita *et al*, 2006) and suggests that it is produced, at least in part, through enhancement of glutamatergic neurotransmission in the prefrontal-accumbal pathway. In addition, nisoxetine might also activate other glutamatergic projections to the NAc, including those from the BLA and vHPC (Grace *et al*, 2007).

In support of this conclusion, c-Fos activation produced by systemic treatment with nisoxetine was observed in the mPFc, NAc shell, BLA, and vHPC in both WT and DAT KO mice, all areas known to be important for the regulation of PPI. In most brain areas assessed there was no basal expression of c-Fos, consistent with previous observations (Sumner *et al*, 2004). Systemic nisoxetine produced substantial increases in c-Fos expression in all of these structures as well as the NAc shell and BLA, which would appear to identify a particular neural circuit activated by nisoxetine, which includes the NAc shell (Hasue and Shammah-Lagnado, 2002) and glutamatergic projections to the NAc shell from the mPFc, BLA, and vHPC (Brog *et al*, 1993; Grace *et al*, 2007). This neuroanatomical circuitry that includes mPFc, BLA, vHPC, and NAc has also been identified as an important portion of the circuitry that regulates PPI (Swerdlow *et al*, 2001; Schmajuk and Larrauri, 2005).

As the next step in identifying the critical site of action of nisoxetine that normalizes PPI in DAT KO mice, the consequences of local intracerebral administration of nisoxetine on PPI was examined. Based on prior data and

c-Fos mapping results, the mPFc and NAc were selected for local application of nisoxetine. In addition to the c-Fos study presented here, a number of studies have suggested that the mPFC and NAc are important anatomical sites regulating PPI (Swerdlow *et al*, 2001; Schmajuk and Larrauri, 2005). The mPFC, in particular, might be relevant to the effects of nisoxetine as mPFc dopamine regulates PPI, and NET is the primary mediator of frontocortical dopamine uptake (Carboni *et al*, 1990; Yamamoto and Novotney, 1998; Moron *et al*, 2002). That NET effects upon mPFC dopamine, and not norepinephrine, levels might be most relevant to mPFc regulation of PPI is suggested by the ability of dopamine D1 and D2 antagonists in the PFc to reduce PPI (Ellenbroek *et al*, 1996; Shoemaker *et al*, 2005). Furthermore, systemic nisoxetine enhances mPFc dopamine levels in both WT and DAT KO mice (Arime *et al*, 2006), and atomoxetine increases dopamine in the mPFc, but not the NAc (Bymaster *et al*, 2002). Thus, specific NET blockade by local administration of nisoxetine into the mPFc would be expected to selectively enhance dopamine neurotransmission in the mPFC, making this action of nisoxetine a strong candidate to mediate normalization of PPI in DAT KO mice. Indeed, the present study found that selective NET blockade in the mPFc, but not the NAc, ameliorated PPI deficits in DAT KO mice, confirming that this structure is an important site of action for this effect. That the effects of NET blockade are mediated by mPFC dopamine is also consistent with a recent imaging study that identified altered prefrontal circuitry in DAT KO mice indicative of hypofrontality (Zhang *et al*, 2010). This does not rule out contributions from other structures, and given that c-Fos activation was observed in both the BLA and vHPC, which also have glutamatergic projections to the NAc, these other structures might also contribute to this effect. Previous studies reported that blockade of NMDA receptors in the BLA reduces PPI in rats, that this impairment is reversed by the dopamine D2 receptor antagonist haloperidol (Fendt *et al*, 2000), and that enhanced NMDA receptor activity in the vHPC also reduces PPI (Wan *et al*, 1996a). The involvement of amygdalo-accumbens or subiculo-accumbens glutamatergic neurotransmission *via* NMDA receptors in PPI regulation suggests that NRIs might ameliorate PPI deficits *via* activation of these subcortical glutamatergic pathways in addition to the prefrontal-accumbens pathway. However, it is known that there are species differences in the regulation of PPI during systemic administration of dopamine D1 and D2 agonist (Ralph and Caine, 2005). Therefore, it remains unclear which type of receptors in the mPFc and hippocampus have a role in the amelioration of PPI deficits in DAT KO mice.

Although it would appear likely that nisoxetine effects upon mPFc dopamine function likely contribute to these effects, the present experiments cannot determine whether c-Fos activation, and the consequent effects of these activated mPFc glutamatergic neurons, is in fact mediated primarily by increased dopamine or norepinephrine neurotransmission. Systemic administration of the dopamine D1/D2 agonist apomorphine and local infusion of norepinephrine into the mPFc both increase mPFc c-Fos expression (Stone *et al*, 1991; Deutch and Duman, 1996). Clozapine and olanzapine have been shown to induce c-Fos expression in the mPFc *via* noradrenergic mechanisms (Ohashi *et al*, 2000), and the selective NET blocker atomoxetine also increases mPFc c-Fos immunoreactivity (Bymaster *et al*, 2002). Our immunohistochemical double-labeling results for c-Fos and the glutamatergic cell marker PAG identified co-labeling in pyramidal glutamatergic mPFc neurons in ~90% of c-Fos-positive cells expressing PAG in both WT and DAT KO mice, indicating that these mPFc neurons were indeed activated by nisoxetine (Table 2). Furthermore, triple fluorescence studies for c-Fos, specific neuronal markers, and the retrograde tracer fluorogold indicated that nisoxetine activates mPFc glutamatergic neurons that project to the NAc shell, which receives mPFc projections (Wright and Groenewegen, 1995). Furthermore, dopamine terminals form synapses on these mPFc pyramidal cells (Carr *et al*, 1999). Taken together, these data suggest that glutamatergic inputs from the mPFc to the NAc shell region are modulated by effects of nisoxetine on catecholaminergic inputs to the mPFc, and modulation of this pathway is responsible, at least in part, for the normalization of PPI by nisoxetine in DAT KO mice.

The present results also demonstrated that systemic nisoxetine activates GABAergic neurons in the NAc shell, based on immunohistochemical double-labeling for c-Fos and GAD67, a marker of GABAergic neurons. In the NAc, GABAergic medium spiny neurons constitute ~90% of all neurons (Meredith, 1999). These neurons receive excitatory inputs from the mPFc, BLA, and ventral subiculum (Brog *et al*, 1993; Grace *et al*, 2007), as well as dopamine innervation from the VTA (Margolis *et al*, 2006), and PPI is regulated by both of these projections (Wan and Swerdlow, 1996b; Swerdlow *et al*, 2001). Systemic nisoxetine activated the mPFC, BLA, and ventral subiculum. However, there were no nisoxetine-induced increases in the number of c-Fos-positive cells projecting from the VTA to NAc. Thus, although nisoxetine might inhibit hyperdopaminergic neurotransmission indirectly, there was no evidence for direct actions on dopaminergic neurons. Therefore, it would seem that NAc GABAergic neurons that showed nisoxetine-induced increases in c-Fos expression were secondarily activated by excitatory glutamatergic inputs from other regions, such as the mPFc. These results suggest that nisoxetine enhances glutamatergic signaling in the NAc shell and that subsequent GABAergic activation might also be involved in the normalization of PPI in DAT KO mice.

The behavioral, pharmacological, and anatomical data presented here indicate that the NET inhibitor nisoxetine normalizes PPI deficits in hyperdopaminergic mouse model, and that the mechanism underlying these effects most likely involves activation of glutamatergic neurons that includes, but may not be limited to, those projecting from the mPFc to the NAc. Schizophrenia is associated with alterations in the anatomy and function of several cortical and subcortical brain areas, including those that have been shown to be most relevant to the effects of nisoxetine in the present studies, and are thought to underlie the cognitive impairments observed in schizophrenic patients (Lewis and Lieberman, 2000; Marek *et al*, 2010). PPI is not considered to be a cognitive measure *per se* but such abnormalities in preattentive information processing might be predictive of cognitive deficits (Geyer, 2006). Our results suggest that selective NRIs, such as nisoxetine, might improve

information processing deficits in schizophrenia *via* regulation of this malfunctioning cortico-subcortical and mesolimbic circuitry.

ACKNOWLEDGEMENTS

We thank Dr Takeshi Kaneko for the gift of PAG monoclonal antibody, Nozomi Okayasu for technical assistance, and Dr Taku Sato and Dr Shiho Miyazawa for assistance with statistical analysis. This work was supported in part by Scientific Research on Priority Areas-System study on higher-order brain functions and Research on Pathomechanisms of Brain Disorders, Core Research for Evolutional Science and Technology (CREST) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Global COE Program (Basic and Translational Research Center for Global Brain Science), MEXT, Japan, and through funding from the Intramural Research Program of the National Institute on Drug Abuse, NIH/DHHS, USA (GRU and FSH).

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

Arime Y, Yamashita M, Fukushima S, Shen H-W, Hagino Y, Hall FS *et al* (2006). Norepinephrine transporter blockade reversed the prepulse inhibition deficits in dopamine transporter knockout mice. *The Society for Neuroscience 36th Annual Meeting*, Atlanta, USA.

Bisler S, Schleicher A, Gass P, Stehle JH, Zilles K, Staiger JF (2002). Expression of c-Fos, ICER, Krox-24 and JunB in the whisker-to-barrel pathway of rats: time course of induction upon whisker stimulation by tactile exploration of an enriched environment. *J Chem Neuroanat* **23**: 187–198.

Braff DL, Geyer MA, Swerdlow NR (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)* **156**: 234–258.

Brog JS, Salyapongse A, Deutch AY, Zahm DS (1993). The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol* **338**: 255–278.

Bymaster FP, Katner JS, Nelson DL, Hemrick-Luecke SK, Threlkeld PG, Heiligenstein JH *et al* (2002). Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology* **27**: 699–711.

Carboni E, Tanda GL, Frau R, Di Chiara G (1990). Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up *in vivo* by noradrenergic terminals. *J Neurochem* **55**: 1067–1070.

Carr DB, O'Donnell P, Card JP, Sesack SR (1999). Dopamine terminals in the rat prefrontal cortex synapse on pyramidal cells that project to the nucleus accumbens. *J Neurosci* **19**: 11049–11060.

Deutch AY, Duman RS (1996). The effects of antipsychotic drugs on Fos protein expression in the prefrontal cortex: cellular localization and pharmacological characterization. *Neuroscience* **70**: 377–389.

Ellenbroek BA, Budde S, Cools AR (1996). Prepulse inhibition and latent inhibition: the role of dopamine in the medial prefrontal cortex. *Neuroscience* **75**: 535–542.

Fendt M, Schwienbacher I, Koch M (2000). Amygdaloid N-methyl-D-aspartate and gamma-aminobutyric acid(A) receptors regulate sensorimotor gating in a dopamine-dependent way in rats. *Neuroscience* **98**: 55–60.

Gehlert DR, Thompson LK, Hemrick-Luecke SK, Shaw J (2008). Monoaminergic compensation in the neuropeptide Y deficient mouse brain. *Neuropeptides* **42**: 367–375.

Geyer MA (2006). Are cross-species measures of sensorimotor gating useful for the discovery of procognitive cotreatments for schizophrenia? *Dialogues Clin Neurosci* **8**: 9–16.

Geyer MA, Dulawa SC (2003). Assessment of murine startle reactivity, prepulse inhibition, and habituation. *Curr Protoc Neurosci* **Chapter 8**, Unit 8 17.

Goldberg TE, Goldman RS, Burdick KE, Malhotra AK, Lencz T, Patel RC *et al* (2007). Cognitive improvement after treatment with second-generation antipsychotic medications in first-episode schizophrenia: is it a practice effect? *Arch Gen Psychiatry* **64**: 1115–1122.

Grace AA, Floresco SB, Goto Y, Lodge DJ (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends Neurosci* **30**: 220–227.

Hasue RH, Shammah-Lagnado SJ (2002). Origin of the dopaminergic innervation of the central extended amygdala and accumbens shell: a combined retrograde tracing and immunohistochemical study in the rat. *J Comp Neurol* **454**: 15–33.

Ishii D, Matsuzawa D, Kanahara N, Matsuda S, Sutoh C, Ohtsuka H *et al* (2010). Effects of aripiprazole on MK-801-induced prepulse inhibition deficits and mitogen-activated protein kinase signal transduction pathway. *Neurosci Lett* **471**: 53–57.

Iversen SD, Iversen LL (2007). Dopamine: 50 years in perspective. *Trends Neurosci* **30**: 188–193.

Jentsch JD, Roth RH (1999). The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* **20**: 201–225.

Keefe RS, Bilder RM, Davis SM, Harvey PD, Palmer BW, Gold JM *et al* (2007). Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE trial. *Arch Gen Psychiatry* **64**: 633–647.

Lewis DA, Lieberman JA (2000). Catching up on schizophrenia: natural history and neurobiology. *Neuron* **28**: 325–334.

Marek GJ, Behl B, Bespalov AY, Gross G, Lee Y, Schoemaker H (2010). Glutamatergic (N-methyl-D-aspartate receptor) hypofrontality in schizophrenia: too little juice or a miswired brain? *Mol Pharmacol* **77**: 317–326.

Margolis EB, Lock H, Chefer VI, Shippenberg TS, Hjelmstad GO, Fields HL (2006). Kappa opioids selectively control dopaminergic neurons projecting to the prefrontal cortex. *Proc Natl Acad Sci USA* **103**: 2938–2942.

Meredith GE (1999). The synaptic framework for chemical signaling in nucleus accumbens. *Ann N Y Acad Sci* **877**: 140–156.

Moron JA, Brockington A, Wise RA, Rocha BA, Hope BT (2002). Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. *J Neurosci* **22**: 389–395.

Mueser KT, McGurk SR (2004). Schizophrenia. *Lancet* **363**: 2063–2072.

Ohashi K, Hamamura T, Lee Y, Fujiwara Y, Suzuki H, Kuroda S (2000). Clozapine- and olanzapine-induced Fos expression in the rat medial prefrontal cortex is mediated by beta-adrenoceptors. *Neuropsychopharmacology* **23**: 162–169.

Paxinos GFK (2001). *The mouse brain in stereotaxic coordinates* 2nd edn. Academic Press: San Diego.

Ralph RJ, Caine SB (2005). Dopamine D1 and D2 agonist effects on prepulse inhibition and locomotion: comparison of Sprague-Dawley rats to Swiss-Webster, 129X1/SvJ, C57BL/6J, and DBA/2J mice. *J Pharmacol Exp Ther* **312**: 733–741.

Ralph RJ, Paulus MP, Fumagalli F, Caron MG, Geyer MA (2001). Prepulse inhibition deficits and perseverative motor patterns in dopamine transporter knock-out mice: differential effects of D1 and D2 receptor antagonists. *J Neurosci* **21**: 305–313.

Schmajuk NA, Larrauri JA (2005). Neural network model of prepulse inhibition. *Behav Neurosci* **119**: 1546–1562.

Schroeter S, Apparsundaram S, Wiley RG, Miner LH, Sesack SR, Blakely RD (2000). Immunolocalization of the cocaine- and antidepressant-sensitive l-norepinephrine transporter. *J Comp Neurol* **420**: 211–232.

Shen HW, Hagino Y, Kobayashi H, Shinohara-Tanaka K, Ikeda K, Yamamoto H *et al* (2004). Regional differences in extracellular dopamine and serotonin assessed by *in vivo* microdialysis in mice lacking dopamine and/or serotonin transporters. *Neuropsychopharmacology* **29**: 1790–1799.

Shoemaker JM, Saint Marie RL, Bongiovanni MJ, Neary AC, Tochen LS, Swerdlow NR (2005). Prefrontal D1 and ventral hippocampal N-methyl-D-aspartate regulation of startle gating in rats. *Neuroscience* **135**: 385–394.

Sora I, Wichems C, Takahashi N, Li XF, Zeng Z, Revay R *et al* (1998). Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. *Proc Natl Acad Sci USA* **95**: 7699–7704.

Stone EA, Zhang Y, John SM, Bing G (1991). c-Fos response to administration of catecholamines into brain by microdialysis. *Neurosci Lett* **133**: 33–35.

Sumner BE, Cruise LA, Slattery DA, Hill DR, Shahid M, Henry B (2004). Testing the validity of c-fos expression profiling to aid the therapeutic classification of psychoactive drugs. *Psychopharmacology (Berl)* **171**: 306–321.

Swerdlow NR, Geyer MA, Braff DL (2001). Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology (Berl)* **156**: 194–215.

Swerdlow NR, Light GA, Cadenhead KS, Sprock J, Hsieh MH, Braff DL (2006). Startle gating deficits in a large cohort of patients with schizophrenia: relationship to medications, symptoms, neurocognition, and level of function. *Arch Gen Psychiatry* **63**: 1325–1335.

Tamminga CA (2006). The neurobiology of cognition in schizophrenia. *J Clin Psychiatry* **67**(Suppl 9): 9–13 discussion 36–42.

Wan FJ, Caine SB, Swerdlow NR (1996a). The ventral subiculum modulation of prepulse inhibition is not mediated via dopamine D2 or nucleus accumbens non-NMDA glutamate receptor activity. *Eur J Pharmacol* **314**: 9–18.

Wan FJ, Swerdlow NR (1996b). Sensorimotor gating in rats is regulated by different dopamine-glutamate interactions in the nucleus accumbens core and shell subregions. *Brain Res* **722**: 168–176.

Wright CI, Groenewegen HJ (1995). Patterns of convergence and segregation in the medial nucleus accumbens of the rat: relationships of prefrontal cortical, midline thalamic, and basal amygdaloid afferents. *J Comp Neurol* **361**: 383–403.

Yamamoto BK, Novotney S (1998). Regulation of extracellular dopamine by the norepinephrine transporter. *J Neurochem* **71**: 274–280.

Yamashita M, Fukushima S, Shen HW, Hall FS, Uhl GR, Numachi Y *et al* (2006). Norepinephrine transporter blockade can normalize the prepulse inhibition deficits found in dopamine transporter knockout mice. *Neuropsychopharmacology* **31**: 2132–2139.

Zhang X, Bearer EL, Boulat B, Hall FS, Uhl GR, Jacobs RE (2010). Altered neurocircuitry in the dopamine transporter knockout mouse brain. *PLoS One* **5**: e11506.

脳に働く〝くすり〟のメカニズム

榊原泰史・曽良一郎

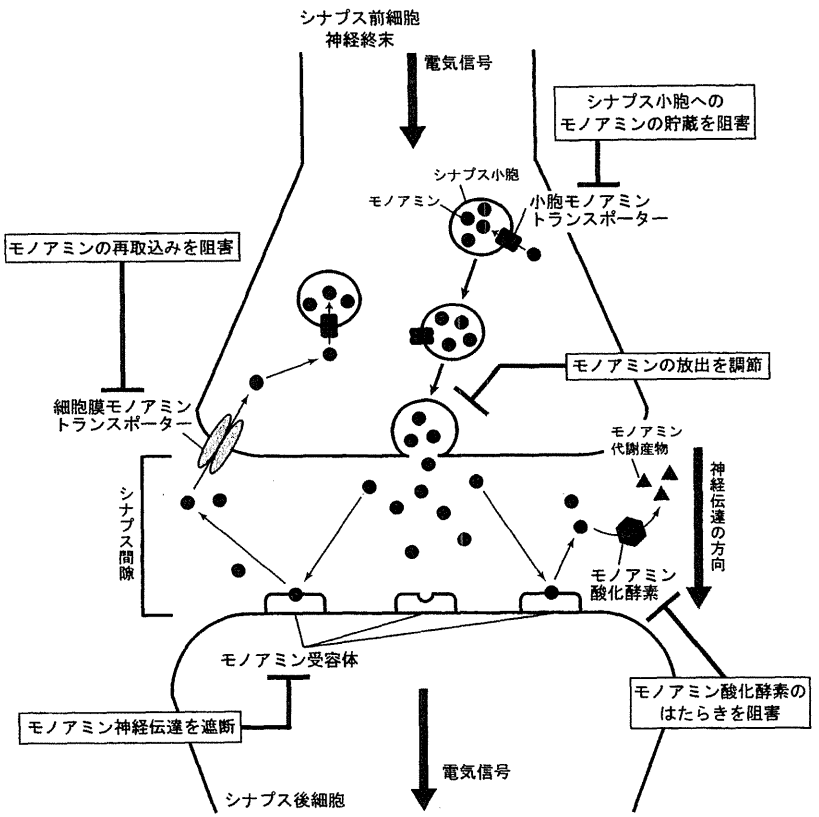
さかきばら・やすふみ
東北大学大学院医学系研究科精神・神経生物学分野博士課程在籍。専攻は神経科学。東北大学大学院生命科学研究科修士課程修了。
そら・いちろう
東北大学大学院医学系研究科精神・神経生物学分野教授。医学博士。専門は精神神経薬理学。岡山大学大学院修了。米国NIH薬物依存研究所分子遺伝学研究室長、東京都精神研・分子精神医学研究部門長を経て現職。自然科学研究機構生理学研究所客員教授、厚生労働省薬事・食品衛生審議会委員、医薬品医療機器総合機構（PMDA）専門委員などを務める。

脳に働きかけて人間の精神機能に影響を及ぼす薬物は、「向精神薬」と総称される。広義の向精神薬には、精神科で処方されるような精神安定剤や抗うつ薬、手術の際に用いられる麻酔薬のほかに、麻薬や覚せい剤などの違法薬物も含まれる。向精神薬の作用機序（作用の仕組み）はさまざまであるが、主に脳内の神経伝達系に作用してそれらを変化させることで、その効果を発揮するものが多い。本稿では、脳内の神経伝達系の一つであるモノアミン神経伝達に焦点を当て、脳に働く向精神薬のメカニズムについて概説したい。

神経伝達の仕組みとモノアミン

ヒトの脳にはおよそ一〇〇〇億個以上の神経細胞（ニューロン）が存在しており、それらは互いにつながり合い、複雑なネットワークを形成している。一つの神経細胞の中では、情報は電気信号という形で伝えられるが、他の神経細胞に電気信号のまま情報を伝達することはできない。なぜなら、神経細胞同士のつなぎ目である「シナプス」と呼ばれる部位にはわずかに隙

図1　シナプスにおけるモノアミン神経伝達と向精神薬の作用



向精神薬の作用標的としては、細胞膜モノアミントランスポーターと小胞モノアミントランスポーター、モノアミン受容体、モノアミン酸化酵素などがある。多くの向精神薬は、これらの働きを調節することで、神経伝達を増強、あるいは抑制する。

間（シナプス間隙）が空いており、電気信号はこの隙間を飛び越えることはできないからである。そこで、送り手側の神経細胞（シナプス前細胞）はこの電気信号に代わり、「神経伝達物質」という化学物質を使って情報を受け手側の神経細胞（シナプス後細胞）に伝達しているのである。

神経伝達物質はこれまでに数十種類が見つかっており、その中でも重要な一群が「モノアミン」と呼ばれる物質である。主なモノアミンとして、ドーパミン、ノルエピネフリン